BEST AVAILABLE IMAGES

Defective images within this document are accurate representation of The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

REF 1

THE RETINOIDS: Biology, Chemistry, and Medicine, 2nd edition. Edited by M. B. Sporn, A. B. Roberts, and D. S. Goodman. Raven Press, Ltd., New York, 1994.

16 / Synthetic Retinoids in Dermatology

Gary L. Peck and John J. DiGiovanna

INTRODUCTION

During the past decade, two synthetic retinoids have been introduced into the clinical practice of dermatology. Isotretinoin [13-*cis*-retinoic acid (Accutane)] is widely used for the treatment of severe cystic acne, and etretinate (Tegison) is used either alone or in combination with other agents for the treatment of psoriasis, particularly the erythrodermic and pustular varieties. In addition, these retinoids are effective in the treatment of many cutaneous disorders of keratinization. In many of these diseases, etretinate is more effective than isotretinoin. Evidence in several reports indicates that synthetic retinoids are effective in preventing skin cancer, and to a lesser extent in its treatment. Isotretinoin and etretinate differ not only in their spectra of clinical efficacy, but also in their observed toxicities and pharmacokinetics. This indicates that each retinoid should be studied as a unique drug, and that the lack of a disease response to one retinoid does not equate with unresponsiveness to all retinoids.

In addition to the synthetic retinoids, a naturally occurring metabolite of retinol, all-*trans*-retinoic acid (RA; tretinoin), has been used clinically in the treatment of several dermatoses (Stuttgen, 1975; Kligman et al., 1969), and is currently accepted as standard topical therapy for acne vulgaris. Early experience with the systemic use of tretinoin was limited by its toxicity (Stuttgen, 1975). However, its recent use as a

G. L. Peck: Department of Dermatology, University of Maryland School of Medicine, Baltimore, MD 21201.
J. J. DiGiovanna: Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Portions of this chapter are reprinted from: Peck, G. L., (1993): The Retinoids. In: *Dermatology in General Medicine*, 4th ed., edited by T. Fitzpatrick et al., with permission of McGraw-Hill.

differentiation-inducing agent for acute promyelocytic leukemia has led to renewed interest in this retinoid (Breitman et al., 1980; Huang et al., 1988).

The discovery of these agents represents the culmination of a decade of clinical and laboratory investigation into the therapeutic spectrum and mechanisms of action of retinoids in the skin. Dermatologic interest in retinoids arose 50 years ago from the use of oral vitamin A as therapy for a variety of dermatoses. This interest was initially based on: (1) the similarity of the follicular keratoses seen in vitamin A deficiency to those in Darier's disease (keratosis follicularis) and pityriasis rubra pilaris; and (2) reports of low levels of serum retinol in these conditions (Peck et al., 1941; Porter, 1951).

Vitamin A deficiency provides a conceptual link in understanding how retinoids may be effective as treatment agents for a wide range of dermatologic disorders. Vitamin A deficiency is characterized by squamous metaplasia of a variety of epithelia, with increased cell proliferation and hyperkeratosis. These changes are also features of some benign dermatoses, such as psoriasis. Once the beneficial effects of oral vitamin A had been observed, its use spread to the treatment of other diseases of the epidermis and epidermal appendages, including acne and basal cell carcinoma. Since the syndrome of hypervitaminosis A interfered with long-term treatment with vitamin A, the need arose for synthetic derivatives that could be at least as efficacious as vitamin A and yet be less toxic. The uses of isotretinoin and etretinate, as described above, represent the initial developments of this concept in clinical practice. With the dramatic response of several previously treatment-resistant dermatoses, and the wide spectrum of benign and malignant conditions noted to be at least partially responsive, the retinoids have ushered in a new era in the therapy of skin disease.

BIOLOGIC EFFECTS OF RETINOIDS ON THE SKIN

The general mechanisms of retinoid action relevant to the use of these substances in dermatology are considered at great length elsewhere in this book, and will not be discussed further here. The reader should consult the various chapters dealing with cellular retinoid-binding proteins, nuclear receptors for retinoids, and the cellular biology and biochemistry of retinoids for specific details.

Epidermal Differentiation (Keratinization)

Transport of Retinoids to the Epidermis

The exposure of the epidermis to a retinoid depends not only on the retinoid concentration in the plasma but also on the tissue penetration, cellular uptake, and intracellular metabolism of the retinoid (Vahlquist and Torma, 1988). The release of retinol from its physiologic transport protein, retinol-binding protein (RBP), is probably mediated by cell-surface receptors that recognize RBP and have been demonstrated in human epidermis (Torma and Vahlquist, 1984).

Metabolism of Retinoids in the Epidermis

Although the active tissue metabolites and the mechanisms of degradation and removal of many retinoids from epithelial tissues are unknown, a number of retinoid metabolites have been identified. In human epidermis, retinol is converted to 3,4-didehydroretinol. This metabolite has been observed to accumulate in psoriasis and several other disorders of keratinization (Vahlquist and Torma, 1988). Isotretinoin, but not aromatic retinoids such as etretinate and acitretin, inhibits this conversion to 3,4-didehydroretinol in epidermis and, more extensively, in sebaceous glands (Vahlquist and Torma, 1991). A small amount of retinoic acid (RA) is formed from retinol in tissues, including the epidermis. This RA is quickly converted to other metabolites (Zile, 1980; Connor and Smit, 1987). The formation of RA from retinol is greater in differentiating than in nondifferentiated keratinocytes, and is greater in the hyperproliferative epidermis of the psoriatic plaque than in normal human skin (Siegenthaler and Saurat, 1991). Higher levels of cellular retinol-binding protein (CRABP) and of nuclear retinoic acid receptors (RARs) have also been observed in differentiating keratinocytes. In addition to the all-*trans* form of RA, 13-*cis*-RA (isotretinoin) is a naturally occurring metabolite of retinol that is only present in tissues in small quantities (Frolik, 1981). Metabolic products of isotretinoin have also been identified. The identification of a series of nuclear receptors for the retinoids raises the possibility that a variety of retinoid metabolites may interact with different receptors. These interactions may vary between different tissues.

Morphologic Effects

Since Fell and Mellanby (1953) initially observed the inhibition of keratinization in chick embryo skin and its subsequent transformation into a mucous-secreting structure by retinol, many studies have been concerned with the mode of action of retinoids on a variety of epithelia. The initial effects of retinoids are focal and reversible, selectively altering the differentiation of germinative layers of epithelia.

The morphologic changes observed in cultures of embryonic chick skin exposed to all-*trans*-RA (tretinoin) were reversible and both dose- and time-dependent. Keratinization was inhibited, and fewer desmosomes and tonofilaments were seen. Golgi elements, rough endo-

plasmic reticulum, and polyribosomes were unusually prominent. Mucin granules formed and gland-like structures developed with intercellular canaliculi characterized by tight junctions, brush borders, and dense secretory contents (Peck et al., 1977).

Several possible mechanisms were considered by which RA could alter epidermal differentiation in this system. Retinoic acid-induced gaps in the basal lamina allowed direct contact between epidermal basal cells and fibroblasts and collagen fibers, which could result in inappropriate dermal signals reaching the epidermis. In younger embryos the entire epidermis, including the mitotically inactive surface cells, appeared to respond to RA, which could imply an epigenetic modulation of cell phenotype. Finally, after the formation of a stratum corneum in older embryos, only the relatively undifferentiated basal layer showed a metaplastic response, indicating that RA could be acting directly on the genome (Peck et al., 1977). The ability of retinoids to exert their effects directly on the genome is supported by the upregulation of the gene for RAR-β, as documented in RA-induced glandular metaplasia of embryonic mouse lip vibrissae (Viallet et al., 1991).

In contrast to their observed effects in embryonic chick skin, retinoids do not produce mucous metaplasia in postembryonic mammalian epidermis, although minor metaplastic changes (formation of microvilli, Golgi apparatus, and secretory granules) were observed when rat external-ear-canal explants were treated with a retinoid-supplemented medium (Liauw et al., 1991). However, both oral and topical retinoids profoundly affect epidermal morphology. Topical RA enhances cell proliferation and hyperplasia, leading to epidermal acanthosis. Initially, after the application of both topical and oral retinoids, there is diminution of the granular layer, but with long-term administration, pronounced hypergranulosis occurs (Elias and Williams, 1981). Retinoid treatment decreases the cohesiveness of the stratum corneum, with resultant impaired function of the permeability barrier and increased transepidermal water loss and enhanced fragility of the upper epidermis (Elias et al., 1981a). This interference with permeability-barrier function enhances the percutaneous absorption of topical agents, which may be either therapeutically beneficial or potentially toxic from unsuspected drug absorption (Fritsch et al., 1981).

Ultrastructurally, retinoid treatment of the epidermis results in decreased numbers of tonofilaments and desmosomal attachments; the emergence of tight junctions; an increased number of keratinosomes, mitochondria, ribosomes, and endoplasmic reticulum; and an increased amount of amorphous material that is seen within widened intercellular spaces (Fritsch, 1981; Williams and Elias, 1981).

The histologic effects of etretinate in psoriatic skin included regression of the inflammatory cell infiltrate, the extracellular accumulation of an amorphous material considered by some to be mucus-like, an increase in the size and number of keratohyalin granules, a widened intercellular space, the reappearance of the stratum corneum layer where it had been diminished, and the regression to normal of other pathologic changes, not only in the epidermis but also in the dermis, particularly the appearance of the capillaries of the dermal papillae (Ward et al., 1983).

The histologic changes in hairless mice receiving etretinate and in patients with disorders of keratinization treated with isotretinoin were similar. The stratum corneum appeared loose, disorganized, and fragmented, and demonstrated patchy areas of reduced thickness. Desmosomes were lost as a result of shedding of these structures at the level of the stratum spinosum (Williams and Elias, 1981). Consequently, desmosomes were absent from many regions in the stratum granulosum. Perinuclear and intercellular deposits of amorphous material that did not stain with mucin stains were present.

A dramatic reduction in the size and number of desmosomes was observed in a freeze-fracture analysis of keratinocyte membranes in patients with psoriasis treated with etretinate (Kitajima and Mori, 1983). This reduction in desmosomal size and number (Williams and Elias, 1981), which was particularly evident in the stratum corneum but was also observed in the stratum spinosum of both lesional and nonlesional skin, appeared to be of sufficient magnitude to contribute significantly to the keratolytic effect of retinoids in hyperkeratotic disorders. Other factors, such as decreased tonofilaments and decreased glycocalyx cohesion, may also contribute to this effect. This keratolytic effect has also been observed *in vitro*, since retinoids cause increased shedding of squames from stratified cultures of human foreskin epidermal cells (McGuire et al., 1982).

Treatment with oral retinoids induces fine granular, amorphous, mucus-like deposits within the epidermis. The nature of these deposits remains unclear. They appear both within and between keratinocytes in the upper stratum spinosum, and persist into the stratum corneum. Through freeze-fracture analysis, this amorphous material was found to be associated with the openings of plasma-membrane vesicles. Transmission electron microscopy showed no evidence of active secretion or endocytosis. The amorphous material did not stain with the periodic acid-Schiff (PAS) or Alcian blue stains or with fluorescent lectins, and was thereby thought unlikely to be mucin or another glycoprotein (Williams and Elias, 1981). Therefore, the formation of these deposits is unlikely to represent mucous metaplasia. Moreover, mucous metaplasia could not be detected by lectin staining, even when hairless mice were treated with a high dose of etretinate, such as 50 mg/kg/d.

Lectin staining of mammalian epidermis reveals a pattern of increased sugar complexity during normal kera-

tinocyte maturation. Retinoids can disrupt this pattern without producing mucous metaplasia, but only at high doses and late in the course of treatment. For example, in etretinate-treated hairless mice, abnormalities in lectin staining developed only after 15 days of therapy. By that time dramatic alterations in epidermal structure and function had already occurred, including abnormal transepidermal water loss, mild acanthosis, and focal loss of stratification (Nemanic et al., 1982; Elias et al., 1983).

Both the accumulation of amorphous material and the diminution of desmosomes induced by retinoids seemed to be responsible for the enhanced fragility of the upper epidermis to frictional trauma. This enhanced fragility led to an intraepidermal cleavage plane that traversed intercellular spaces filled with the amorphous material and desmosome-depleted surfaces. Since keratinocytes containing these amorphous deposits seemed to be fragile, the intercellular deposits may have accumulated as a result of the rupture and leakage of cells containing the amorphous material. Additionally, it has been suggested that this amorphous material could be secondary to serum infiltration into the epidermis, with macropinocytosis accounting for the intracellular localization of the material (Ellis et al., 1982).

Epidermal Cell Proliferation, Polyamines, Ornithine Decarboxylase

The changes in cell-kinetic parameters induced in hairless mice by etretinate (1–10 mg/kg/d) were dose-dependent and consisted of transitory cellular hypertrophy, persistent epidermal hyperplasia, and increased labeling indices. The mean basal cell generation time was greatly accelerated, owing to a shortening of all of the cell-cycle phases that were tested (Fritsch et al., 1981). The fraction of noncycling basal cells was reduced during etretinate therapy. The nuclear enlargement and increase in labeling indices occurred before the onset of retinoid-induced desquamation of the horny layer, indicating that these events were not simply secondary to the mitogenic stimulus that occurs after loss of the stratum corneum, an effect observed after stripping of the skin with adhesive tape.

It may at first seem paradoxical that diseases such as psoriasis and lamellar ichthyosis, which are characterized by a hyperproliferative epidermis, can benefit from drugs such as the retinoids, which can stimulate epidermal proliferation under certain experimental conditions. However, when tested in patients with psoriasis, etretinate led to decreased ornithine decarboxylase activity, decreased levels of urinary and cutaneous polyamines, and decreased epidermal deoxyribonucleic acid (DNA) synthesis (Kaplan et al., 1983).

The concentrations of polyamines and activity of their rate-limiting enzyme ornithine decarboxylase (ODC) are increased in untreated psoriatic skin. Since retinoids were known to interfere with the activity of ODC and with polyamine biosynthesis in experimental cutaneous carcinogenesis, it was anticipated that retinoids would lead to similar effects in psoriasis. Within 4 weeks of etretinate therapy, cutaneous levels of ODC activity and polyamines were significantly reduced in both the diseased and uninvolved skin of patients with psoriasis (Lowe et al., 1982; Lauharanta et al., 1981a; Kaplan et al., 1983). The accelerated polyamine biosynthesis in psoriasis was normalized prior to any significant inhibition of epidermal DNA synthesis. Oral etretinate also led to a dose-dependent decrease in the urinary excretion of polyamines in psoriasis patients. Mean putrescine levels had fallen by 27%, those of spermidine by 34%, and those of spermine by 37% at the end of a 16-week treatment period, indicating an inhibition of polyamine biosynthesis (Grekin et al., 1983). It is known that clinical improvement in psoriasis, by whatever therapy, reduces the cutaneous and urinary levels of polyamines, suggesting that these changes are secondary to disease improvement.

Epidermal Transglutaminase Activity and Cornified Envelope Formation

Transglutaminase is a calcium-dependent, cytosolic enzyme that catalyzes the formation of the (γ-glutamyllysine) dipeptide bond in the cross-linked, cornified envelope in epidermal cells, and is therefore a critical regulator of epidermal differentiation. During terminal differentiation of the epidermis, membrane-bound, keratinocyte-specific, epidermal (type I) transglutaminase-K catalyzes the cross-linking of involucrin and other proteins (loricrin, keratolinin) to form the cornified envelope. Several reports indicate that retinoids inhibit transglutaminase activity and formation of the cornified envelope. For example, RA at physiologic concentrations completely blocks the synthesis of messenger ribonucleic acid (mRNA) for loricrin (Hohl et al., 1991). Retinyl acetate suppresses the ability of cultured keratinocytes, derived from a human squamous-cell carcinoma, to form cross-linked envelopes at the cell periphery (Rice et al., 1983). Cell-envelope protein production was also inhibited by the retinoidal benzoic acid derivative (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB) ethyl ester (Stadler et al., 1984). Another mechanism by which RA interferes with cornification is by suppressing the expression of epidermal transglutaminase (Floyd and Jetten, 1989). In keratinocyte cell cultures, RA markedly reduces the immunostaining and enzyme activity of transglutaminase-K (Griffiths et al., 1992). In contrast, RA cream, when applied to normal human skin *in vivo* for either 4 days under occlusion or 4 months in the treatment of photodamaged skin, increases the protein

plasmic reticulum, and polyribosomes were unusually prominent. Mucin granules formed and gland-like structures developed with intercellular canaliculi characterized by tight junctions, brush borders, and dense secretory contents (Peck et al., 1977).

Several possible mechanisms were considered by which RA could alter epidermal differentiation in this system. Retinoic acid-induced gaps in the basal lamina allowed direct contact between epidermal basal cells and fibroblasts and collagen fibers, which could result in inappropriate dermal signals reaching the epidermis. In younger embryos the entire epidermis, including the mitotically inactive surface cells, appeared to respond to RA, which could imply an epigenetic modulation of cell phenotype. Finally, after the formation of a stratum corneum in older embryos, only the relatively undifferentiated basal layer showed a metaplastic response, indicating that RA could be acting directly on the genome (Peck et al., 1977). The ability of retinoids to exert their effects directly on the genome is supported by the upregulation of the gene for RAR-β, as documented in RA-induced glandular metaplasia of embryonic mouse lip vibrissae (Viallet et al., 1991).

In contrast to their observed effects in embryonic chick skin, retinoids do not produce mucous metaplasia in postembryonic mammalian epidermis, although minor metaplastic changes (formation of microvilli, Golgi apparatus, and secretory granules) were observed when rat external-ear-canal explants were treated with a retinoid-supplemented medium (Liauw et al., 1991). However, both oral and topical retinoids profoundly affect epidermal morphology. Topical RA enhances cell proliferation and hyperplasia, leading to epidermal acanthosis. Initially, after the application of both topical and oral retinoids, there is diminution of the granular layer, but with long-term administration, pronounced hypergranulosis occurs (Elias and Williams, 1981). Retinoid treatment decreases the cohesiveness of the stratum corneum, with resultant impaired function of the permeability barrier and increased transepidermal water loss and enhanced fragility of the upper epidermis (Elias et al., 1981a). This interference with permeability-barrier function enhances the percutaneous absorption of topical agents, which may be either therapeutically beneficial or potentially toxic from unsuspected drug absorption (Fritsch et al., 1981).

extracellular accumulation of an amorphous material considered by some to be mucus-like, an increase in the size and number of keratohyalin granules, a widened intercellular space, the reappearance of the stratum corneum layer where it had been diminished, and the regression to normal of other pathologic changes, not only in the epidermis but also in the dermis, particularly the appearance of the capillaries of the dermal papillae (Ward et al., 1983).

The histologic changes in hairless mice receiving etretinate and in patients with disorders of keratinization treated with isotretinoin were similar. The stratum corneum appeared loose, disorganized, and fragmented, and demonstrated patchy areas of reduced thickness. Desmosomes were lost as a result of shedding of these structures at the level of the stratum spinosum (Williams and Elias, 1981). Consequently, desmosomes were absent from many regions in the stratum granulosum. Perinuclear and intercellular deposits of amorphous material that did not stain with mucin stains were present.

A dramatic reduction in the size and number of desmosomes was observed in a freeze-fracture analysis of keratinocyte membranes in patients with psoriasis treated with etretinate (Kitajima and Mori, 1983). This reduction in desmosomal size and number (Williams and Elias, 1981), which was particularly evident in the stratum corneum but was also observed in the stratum spinosum of both lesional and nonlesional skin, appeared to be of sufficient magnitude to contribute significantly to the keratolytic effect of retinoids in hyperkeratotic disorders. Other factors, such as decreased tonofilaments and decreased glycocalyx cohesion, may also contribute to this effect. This keratolytic effect has also been observed *in vitro*, since retinoids cause increased shedding of squames from stratified cultures of human foreskin epidermal cells (McGuire et al., 1982).

Treatment with oral retinoids induces fine granular, amorphous, mucus-like deposits within the epidermis. The nature of these deposits remains unclear. They appear both within and between keratinocytes in the upper stratum spinosum, and persist into the stratum corneum. Through freeze-fracture analysis, this amorphous material was found to be associated with the openings of plasma-membrane vesicles. Transmission electron microscopy showed no evidence of active secretion or endocytosis. The amorphous material [illegible]

expression and enzymatic activity of transglutaminase-K without increasing its mRNA levels. This indicates a lack of correlation between *in vitro* and *in vivo* findings with regard to effects of RA on the epidermis, possibly owing to the presence in human skin of cytokines or growth factors that could modify the effects of RA. In other studies, retinoids inhibited the activity of transglutaminase type I in squamous cell carcinoma cells of the head and neck and in tracheobronchial epithelial cells (Poddar et al., 1991; Nervi et al., 1991), but led to in-

Cyclic Adenosine Monophosphate, Protein Kinases, Epidermal Growth Factor

Retinoids have effects on epidermal proliferation and keratinization. It is well known that agents that increase the cellular concentration of cyclic AMP (cAMP) also increase epidermal cell proliferation, and that pretreatment with RA enhances this effect (Vahlquist and Torma, 1988). This activity may be mediated by cAMP-dependent protein kinases whose activity is increased

rather than interfering with the initiation phase of carcinogenesis, retinoids act later, during the promotion phase. Accordingly, retinoids are considered "antipromoting" agents. Tumor promoters, such as phorbol esters, the active agents in croton oil, increase the activity of ODC at an early phase of experimental carcinogenesis in skin (O'Brien, 1976; O'Brien et al., 1975). Ornithine decarboxylase is the rate-limiting enzyme in the synthesis of polyamines, which are involved in cell proliferation and differentiation. Retinoids interfere with the ability of phorbol esters to induce ODC (Verma and Boutwell, 1977). The degree to which they inhibit ODC was found to correlate with the ability of a particular retinoid to inhibit the development of skin papillomas (Verma et al., 1979).

Retinoids have a wide variety of effects on malignant transformation *in vitro*, reversing or suppressing transformation caused by chemical carcinogens, ionizing radiation, and transforming peptides such as sarcoma growth factor. Retinoids also interfere with virus-induced transformation, as evidenced by the effectiveness of etretinate in lesions of epidermodysplasia verruciformis, induced by the oncogenic human papilloma viruses (Lutzner and Blanchet-Bardon, 1980).

Membrane Alterations

Retinoids can induce cell-surface alterations that often result in increased adhesiveness of the treated cells (Jetten et al., 1979; Hassell et al., 1979). Retinoids can alter membrane microviscosity and may interact directly with membranes (Jetten, 1984). Another direct effect of retinoids on cell membranes is the production of gap junctions that allow cell-to-cell coupling and facilitate intercellular communication (Elias et al., 1981a; Prutkin, 1975).

On a molecular level, cells exposed to retinoids develop alterations in their surface-protein profiles (Hassell et al., 1979; Lotan et al., 1980), increased protein glycosylation (DeLuca, 1977), modulated glycosaminoglycan synthesis (Jetten et al., 1979; Shapiro and Poon, 1979), and quantitative changes in the protein receptors on their surfaces (Jetten, 1984).

Lysosomes

Much of the early research on retinol emphasized its detergent-like effect on cell membranes. Free retinol, possibly through insertion into cell membranes, can have a detergent-like effect that alters membrane microviscosity (Jetten, 1984). In the blood, the binding of retinol to its transport protein, retinol-binding protein (RBP), minimizes this effect. One result of this detergent-like effect is the labilization of lysosomal membranes (Wang et al., 1976), which may result in lysosomal enzyme release and subsequent retinoid-induced toxicity (Lazarus et al., 1975). The clinical toxicities of acute hypervitaminosis A are consistent with a detergent-like effect on cell membranes as the result of released lysosomal enzymes. Current research, however, indicates that at therapeutic doses, lysosomal labilization and subsequent cytotoxicity are not responsible for most retinoid effects. For instance, in pityriasis rubra pilaris and in Darier's disease treated with oral isotretinoin, the specific activity of the lysosomal hydrolases cathepsin D and β-glucuronidase decreased significantly. This indicates that clinical remissions of these diseases in patients treated with isotretinoin are not due to an increased intracellular concentration of lysosomal enzymes (Farb et al., 1980). In addition to promoting labilization, retinoids can have other effects on lysosomes. For example, the induction by RA of differentiation in embryonal carcinoma cells correlates with an increase in the synthesis and glycosylation of lysosomal-associated membrane glycoproteins (Amos and Lotan, 1990).

Gap Junctions

Gap junctions are communication links that allow the passage of electrical signals, ions, and molecules between cells. These channels are probably important in the control of tissue organization and growth. During the development of malignancy, the number of gap junctions decreases (Weinstein et al., 1976). In response to retinoids, gap junctions proliferate rapidly in neoplastic and embryonic keratinizing epithelia. The rapidity of the response suggests that retinoids act on these structures through a direct effect (Prutkin, 1975; Elias and Friend, 1976).

Another important effect of retinoid treatment is the stimulation of gap-junction hyperplasia and hypertrophy in the epidermis (Elias and Williams, 1981). This stimulation occurs before other effects are observed, such as tight-junction proliferation. Gap-junction proliferation has been observed *in vitro* in embryonic chick skin exposed to RA, and *in vivo* in rabbit keratoacanthoma and human basal cell carcinoma after the topical application of RA (Elias et al., 1981b). Moreover, RA increases the expression of the transmembrane protein connexin 43, a component of gap junctions, in both suprabasalar epidermal cells and in dermal fibroblasts (Guo et al., 1992).

Keratinocyte Membranes in Psoriasis

The membranes of keratinocytes in psoriasis are abnormal when examined by freeze-fracture analysis. Although etretinate can clinically eliminate psoriasis, it may not completely reverse these membrane abnormali-

ties. Even after the lesions in the disease were clinically resolved, etretinate had not reduced the supranormal intramembranous particle density in the fracture face of the protoplasmic half-layer (P face) of the spinous-cell plasma membrane (Kitajima and Mori, 1983).

Cross-fractures of keratinocytes in the lesional skin following treatment with etretinate revealed an increase in vesicular components, a considerable development of Golgi complex, and a decrease in tonofilaments. Cross-fractures of the cell boundaries showed an irregular appearance, with microvilli and cell processes and widened intercellular spaces after etretinate therapy. The thickness of the granular layer was increased. The granular cells were rich in vesicles and poor in tonofilaments. The frequency of gap junctions is higher in psoriatic skin than in normal skin, particularly in the upper spinous layer. However, no significant changes in the size or frequency of gap junctions were found in psoriatic lesional skin following isotretinoin treatment. Gap junctions were frequently noticed on the basal cell plasma membranes in nonlesional skin after treatment (Kitajima and Mori, 1983).

Glycoconjugate Biosynthesis

During vitamin A deficiency there is a decrease in the biosynthesis of carbohydrate-containing macromolecules in epithelial tissues (DeLuca, 1977, 1978). In vitamin A-deficient animals the synthesis of specific glycoproteins can be stimulated by the addition of retinol (DeLuca, 1978). Changes in cell-surface glycoproteins following treatment with retinol have been related to changes in cell morphology and adhesiveness (Adamo et al., 1979). Retinoids can also modify glycolipid biosynthesis (Patt et al., 1978).

Immune Function

Neutrophils

One of the first cellular events observed after the oral etretinate treatment of psoriasis was the loss of neutrophil migration from dermal capillaries to the epidermis. Oral etretinate also inhibited the migration of neutrophils out of suction-blister bases into overlying skin chambers in normal subjects (Dubertret et al., 1982). Topically applied etretinate at 0.1 mg/ml also inhibited neutrophil migration in this system. However, neutrophil migration is inhibited in psoriasis after clearing of skin lesions, whatever the treatment used.

In addition to the foregoing effects, topical isotretinoin was effective in inhibiting the leukotriene B4-induced migration of polymorphonuclear leukocytes into normal skin (Wozel et al., 1991). This reversible anti-inflammatory effect suggests that retinoids may be of value in treating neutrophil-dependent diseases, in addition to acne and psoriasis. Retinoids also inhibit other discrete polymorphonuclear leukocyte functions *in vitro*. The incubation of neutrophils with retinoids causes a dose-dependent inhibition of oxygen production and chemiluminescence induced by phorbol myristate acetate and other agents, and of degranulation induced by *N*-formyl-methionyl-leucyl-phenylalanine (Fumarulo et al., 1991). Retinoids may exert their anti-inflammatory effects by interacting with neutrophil membranes to inhibit a variety of responses, such as lysosomal enzyme release and superoxide generation (Camisa et al., 1982).

Langerhans Cells

Langerhans cells are known to play a major role in immune reactions in the skin, and may also be involved in regulating epidermal differentiation. The distribution and number of Langerhans cells, as detected by monoclonal antibodies directed against human HLA-DR antigens and OKT6, and by cytochemical staining for ATPase, are altered in skin affected by psoriasis and are corrected after successful therapy with oral etretinate (Haftek et al., 1983). It is not known whether this normalization of Langerhans cell distribution in psoriasis is due to a direct effect of etretinate or represents a secondary effect of correcting the altered epidermal differentiation in the disease. In contrast, other successful therapies in psoriasis, such as the use of ultraviolet light (UVB), psoralen and long-wave ultraviolet light (PUVA), and treatment with topical corticosteroids, deplete Langerhans cells from the skin. Topically applied RA prevents both ultraviolet light and the tumor promoter phorbol acetate from reducing the density of Langerhans cells in the epidermis (Halliday et al., 1992).

Other Immune Effects of Retinoids

Retinoids are generally thought to stimulate humoral and cellular immunity, but immune-inhibitory effects of these agents have also been observed (see Chapter 12 for a more complete discussion). Retinoids can act as adjuvants in immune reactivity enhancing antibody production in response to a variety of antigens, and have diverse effects on cell-mediated immunity. For example, treatment with isotretinoin in men with leukoplakia or Barrett's esophagus led to an increase in peripheral blood T-helper cells but not natural killer (NK) cells (Prabhala et al., 1991). Retinoids also inhibited the antigen-presenting properties of epidermal cells and of dendritic cells (Dupuy et al., 1989; Bedford and Knight, 1989). Part of the antitumor and anti-inflammatory effects of retinoids may be due to these immune effects (Dennert, 1984).

Connective Tissue

Both isotretinoin and tretinoin inhibited collagenase and gelatinase production in skin fibroblast cultures derived from normal skin and from patients with recessive dystrophic epidermolysis bullosa (Bauer et al., 1982). The inhibition of collagenase activity was paralleled by a reduction in immunoreactive enzyme, suggesting that these retinoids act by inhibiting the synthesis or secretion of the enzyme, or both. Because of these findings, retinoids were tested as therapeutic agents for recessive dystrophic epidermolysis bullosa, a disease in which the pathogenesis of blistering is in part related to connective-tissue destruction. Low doses (0.4 mg/kg/d) of isotretinoin were effective in reducing the numbers of bullae observed in three patients with severe recessive dystrophic epidermolysis bullosa; however, higher doses increased the number of bullae owing to the superimposition of mucocutaneous toxicity from isotretinoin (xerosis, pruritus, fragility) on the disease process (Cooper et al., 1985). The inhibition of type IV (basement membrane) collagen by retinoids has been documented in human fibrosarcoma cells (Oikarinen, 1989). The inhibitory effect on collagen synthesis by retinoids acting as lipophilic antioxidants may be due to the inhibition of ascorbate-induced lipid peroxidation, a process essential to normal collagen synthesis (Geesin et al., 1990).

As with other biologic effects of retinoids, their actions on fibroblast function may vary with the cell type and culture conditions, the concentrations employed, the specific retinoid studied, and interactions with other agents such as cytokines. Retinoids inhibit fibroblast proliferation during exponential growth. In contrast, increased cell proliferation and collagen synthesis are noted when growth-inhibited fibroblasts are treated with RA (Varani et al., 1990). Retinoids affect fibroblast chemotaxis indirectly by decreasing the chemoattractive response to agents such as fibronectin and platelet-derived growth factor (PDGF). The effects of retinoids on fibroblasts can be modified by cytokines. For example, the retinoid-induced production of TGF-β in epidermal cells may alter the effects of retinoids on fibroblasts as well as collagen synthesis. In addition to normal fibroblasts, inhibition of collagen synthesis by retinoids has been documented in abnormal or diseased fibroblasts, such as those derived from keloids and hypertrophic scars, and from scleroderma patients (Stumpenhausen et al., 1991).

Proteases and Prostaglandins

Retinoids have been shown to affect proteases. Plasminogen activator cleaves plasminogen to plasmin—the enzyme that catalyzes fibrinolysis. Plasminogen activator may be important in tissue remodeling. The treatment of cell lines with retinoids can increase the secretion of plasminogen activator (Sherman et al., 1976; Wilson and Reich, 1978). Another protease, collagenase, has been observed to be suppressed by retinoids (Brinckerhoff et al., 1980; Bauer et al., 1982). Inhibitory effects of retinoids on prostaglandin production in rheumatoid synovial cells have also been described (Brinckerhoff et al., 1980).

Sebaceous Glands

The effectiveness of retinoids in the treatment of acne may be a function of their effects on sebaceous glands and sebum production. Isotretinoin is the most effective retinoid in treating acne, as well in reducing sebaceous gland size and suppressing sebum production. During the culture of human sebocytes, isotretinoin and tretinoin were found to inhibit cell proliferation and total lipid synthesis (Zouboulis et al., 1991). Although cholesterol synthesis was increased, there was a marked reduction in wax and sterol esters, free fatty acids, and triglycerides. Sebaceous cell differentiation was also modified, with a reduced expression of OM-1, a monoclonal antibody marker of sebocyte differentiation, and alterations in keratin-protein expression. These effects of retinoids are presumably mediated by the nuclear RA receptors present within sebocytes, particularly RAR-α and -γ and RXR-α (Doran et al., 1991). Inhibition of the proliferation of human sebaceous cells *in vitro* may be a useful predictive screen for the clinical efficacy of retinoids. Retinoids clinically efficacious for acne (isotretinoin, tretinoin) are active in this model system, whereas clinically ineffective retinoids (temarotene, etretinate) are inactive (Doran and Shapiro, 1990).

CLINICAL STUDIES

The profound clinical impact of the synthetic retinoids was first observed and described in patients with dermatologic disease (Table 1). Clinical efficacy observed with systemic tretinoin (Thomson and Milne, 1969; Eriksen and Cormane, 1975; Stuttgen, 1975), a natural retinoid, preceded the use of the synthetic retinoids. The development of the synthetic derivatives isotretinoin and etretinate, which had less toxicity than tretinoin, generated interest in studies of a variety of dermatologic diseases. Initially, Orfanos and co-workers obtained equivocal results with isotretinoin in the treatment of psoriasis (Orfanos et al., 1972). Subsequently, etretinate, used alone (Ott and Bollag, 1975) or in combination with topical dithranol (Orfanos and Runne, 1976), was found to be very effective for psoriasis. These findings led to the temporary abandonment of isotretinoin. Interest in isotretinoin was rekindled after the dis-

TABLE 1. *The spectrum of retinoid-responsive diseases*

Acne vulgaris and related acneform diseases
Disorders of keratinization
The ichthyoses
Darier's disease
Pityriasis rubra pilaris
Skin cancer and precancer chemotherapy and chemoprophylaxis
Psoriasis vulgaris and its pustular and erythrodermic variants
Miscellaneous cutaneous diseases
Subcorneal pustular dermatosis
Discoid lupus erythematosus
Reiter's syndrome [with and without AIDS]
Warts
Lichen planus
Cutaneous sarcoidosis

covery that it was effective in the treatment of lamellar ichthyosis and other cutaneous disorders of keratinization (Peck and Yoder, 1976), as well as in producing complete responses with prolonged remissions in patients with previously treatment-resistant cystic and conglobate acne (Peck et al., 1979), and partly effective in the treatment and prevention of basal cell carcinoma (Peck et al., 1978, 1982a). Subsequent to these initial findings, isotretinoin and etretinate, used alone or in combination with other agents, have proven successful in an expanding spectrum of skin diseases, such as lupus erythematosus and cutaneous T-cell lymphoma. After identifying the range of clinical efficacy (Table 1) and elucidating the toxicity (Table 2) of the retinoids in dermatologic diseases, interest in these substances spread to other medical specialties such as rheumatology and oncology.

Cystic Acne

In 1976, patients with disorders of keratinization treated with oral isotretinoin were observed to develop drying and chapping of their facial skin resembling that seen with topical tretinoin. Because of this finding and the historic use of oral vitamin A and topical tretinoin in the treatment of acne, it appeared reasonable to treat acne with oral isotretinoin. In the first clinical trial, 14 patients with previously treatment-resistant cystic acne responded dramatically to isotretinoin, at an average maximum dosage of 2.0 mg/kg body weight/d, and had an 85% mean reduction in lesion counts at the end of the 4-month treatment period (Fig. 1) (Peck et al., 1979). Thirteen of the patients went on to complete clearance of their acne after discontinuation of therapy, indicating that therapy need not be maintained until total improvement is observed. This continued healing was regularly followed by prolonged remissions.

Cystic acne is unique among retinoid-responsive diseases in that most cases of even the greatest severity can be successfully treated with only one 4- or 5-month course of isotretinoin at doses of 0.5 to 2.0 mg/kg body weight/d (Peck et al., 1982b). Only about one-third of acne patients require a second course, and only a few require additional therapy for complete clearance of their disease. Because of the continued healing seen after discontinuing therapy, 2-month treatment-free evaluation periods are useful in determining which patients require additional therapy. Generally, patients with severe

TABLE 2. *Spectrum of retinoid toxicity*

Acute:
Mucocutaneous
Cheilitis
Facial dermatitis
Xerosis with pruritus
Conjunctivitis
Dry nasal mucosa with minor nosebleeds
Stratum corneum fragility (peeling from minor trauma)
Palmoplantar peeling
Hair loss
Dry mouth with thirst
Paronychia; nail plate abnormalities[a]
Stickiness of skin[a]; chills[a]
Phototoxicity and photosensitivity[b]
Inflamed urethral meatus[a]
Corneal opacities[b] (reversible after discontinuation)
Pyogenic granuloma-like lesions in acne[b]
Systemic:
Headache[a]
Arthralgias and myalgias[a]
Teratogenicity (head, ear, heart, thymus abnormalities)
Spontaneous abortion; premature births
Pseudotumor cerebri[b] (headache, papilledema)
Mental depression[b]
Inflammatory bowel disease[b]
Urticaria; vasculitis; erythema nodosum[b]
Idiopathic seizures[b]
Laboratory:
Hyperlipidemia:
Increased triglycerides, VLDL
Increased cholesterol, LDL[a]; decreased HDL[a]
Eruptive xanthoma[b]
Acute hemorrhagic pancreatitis[b]
Elevated liver function tests (transient, minor):
AST, ALT, alkaline phosphatase, LDH, bilirubin
Thrombocytosis[a]; thrombocytopenia[b]; leukopenia
Hyperuricemia with gout[b]; hypercalcemia[b]
Elevated CPK and myalgias after exercise[b]
Chronic:
Mucocutaneous—persistent, post-treatment:
Dry eyes[b], hair thinning[b]
Systemic:
Vertebral abnormalities resembling diffuse idiopathic Skeletal hyperostosis
Osteophyte and bony bridge formation
Anterior spinal ligament calcification
Posterior spinal ligament calcification[b]
Tendon and peripheral ligament calcification
Premature epiphyseal closure
Laboratory—none

[a] Uncommon.
[b] Rare.

cystic acne located predominantly on the trunk require higher doses of isotretinoin of up to 2.0 mg/kg/d, and longer treatment periods than do patients with facial acne.

Although isotretinoin has proved to be the most effective therapy for cystic acne, there is typically a lag period before the onset of the therapeutic effect. The usual time for a 50% decrease in the number of acne nodules and cysts on the face is 8 weeks of therapy, and on the trunk 12 weeks. Of those patients whose acne clears completely, most remain totally free of cysts. Some patients have an occasional cyst or two and varying amounts of papular acne at follow-up examinations.

Relapses sufficient to require further therapy with isotretinoin have been reported in about 10 to 40% of acne patients (Cunliffe and Norris, 1987). The tendency to relapse is dose dependent (i.e., patients treated with 0.1 mg/kg/d have a much greater tendency to relapse than those treated with 1.0 to 2.0 mg/kg/d; Jones et al., 1983; Strauss et al., 1984; Meigel et al., 1983; Plewig et al., 1981). Relapses may also be age dependent, being more frequent in adolescents than in older patients, and may be related to the extent and severity of acne present prior to the initiation of therapy (Chivot and Midoun, 1990; Harms et al., 1986). Cunliffe and co-workers state that the risk of relapse is small if it has not occurred within 3 years of the initial course of therapy (Cunliffe et al., 1991). They also find that relapse is less likely to occur if the sebum secretion rate has been suppressed by at least 80% at the end of isotretinoin therapy. Furthermore, relapses are more likely to occur in those patients whose sebum secretion rate returns to within 10% of its baseline values after the cessation of therapy (Cunliffe and Norris, 1987). Relapses are generally mild; it is unusual for acne at the time of relapse to equal its pretreatment severity. When mild or moderate relapses occur, a trial of conventional acne therapy is often effective. If this fails, then additional treatment with isotretinoin may be indicated.

Current dosage recommendations for isotretinoin, based on the results of early trials involving patients with severe cystic acne, are that 1.0 mg/kg/d be used for 4 or 5 months as an initial course of therapy. Although doses as low as 0.05 mg/kg/d were tested, a higher relapse rate with only a moderate reduction in incidence of side effects was reported with these doses. These data argued against the usefulness of low doses in patients with severe cystic acne. It is possible, however, that patients with less severe forms of facial cystic acne, who are being treated earlier in order to minimize or prevent scarring and its psychosocial consequences (Rubinow et al., 1987), will respond comparably and with less toxicity to a dose level of 0.5 mg/kg/d.

After observing the continuing therapeutic benefit regularly seen after the discontinuation of therapy with isotretinoin, an additional dosage schedule was designed. Comparable therapeutic results could be achieved if high initial doses (1 to 2 mg/kg/d) were given for only 2 weeks and followed by lower doses (0.25 to 0.5 mg/kg/d) for the remainder of a 16-week treatment period. The higher doses (2.0 mg/d followed by 0.5 mg/kg/d) were used for patients with predominantly truncal acne, and the lower doses (1.0 mg/d followed by 0.25 mg/kg/d) for those with facial acne. This high-low dosage schedule was superior to both a 2-week high-dosage schedule followed by placebo, and to a constant low-dosage schedule. Specifically, the constant low-dosage schedule (0.5 mg/kg/d) led to an initial 20% increase in the lesion count at 2 weeks and, at the end of the 16-week treatment period, to only a 50% reduction in lesions. In contrast, the high-low dosage schedule did not increase the mean lesion count at the 2-week observation point, but did reduce acne by 75% at 16 weeks.

Patients who require a second course of therapy for substantial, persistent acne may need higher doses of isotretinoin, such as 1.5 to 2.0 mg/kg/d, for an additional 4- to 6-month course of therapy. This is particularly true for acne of the nuchal region, low back, buttocks, and thighs. Doses exceeding 2.0 mg/kg/d are generally not necessary. On the other hand, when treating patients with mild to moderate acne of the face or trunk that has improved but not cleared after an initial course of therapy with isotretinoin, one may use a trial of conventional therapy prior to reinstating isotretinoin at the usual recommended dosage (1.0 mg/kg/d).

In the initial clinical trials, prior to the marketing of isotretinoin, an increased number of acne cysts were occasionally seen during the first 2 weeks of isotretinoin therapy (Katz et al., 1983). This may have been due to isotretinoin paradoxically and temporarily increasing the number of inflammatory lesions derived from closed comedones, an effect perhaps resembling that seen during initial therapy with topical tretinoin. In some reports, however, the increase in numbers of lesions could have been due to the abrupt discontinuation of previously used, partially effective therapy, such as oral antibiotics, 4 weeks prior to the entry of patients into experimental protocols with isotretinoin. It has been also suggested that early in the course of therapy, pro-inflammatory priming of neutrophils by isotretinoin may exacerbate acne (Perkins et al., 1991). While agreement is lacking on either the effect of isotretinoin dosage on the incidence and severity of initial flare-ups of acne or their treatment, such flare-ups were, in our experience, less severe when higher initial doses of isotretinoin (2.0 mg/kg/d) were used. For severe flare-ups, therapy with isotretinoin may be discontinued and treatment with systemic corticosteroids initiated.

One other uncommon reaction in treating cystic acne with isotretinoin is the evolution of acne cysts, particularly on the trunk, into crusted, pyogenic granuloma-like lesions (Shalita et al., 1983; Exner et al., 1983; Hol-

land et al., 1984). These lesions may occur rarely in severe acne untreated with isotretinoin, but are probably more commonly observed during isotretinoin therapy; they respond readily to debridement of the crusts and either the intralesional injection or topical application of corticosteroids, or to a short course of corticosteroids given systemically. An additional adverse effect may be colonization of the anterior nares with *Staphylococcus aureus* during isotretinoin therapy. The use of an antibiotic ointment on the nares has been suggested for preventing staphylococcal folliculitis and furunculosis, which may occur late in the course of isotretinoin therapy and be confused with a relapse of acne.

In addition to its efficacy in cystic acne, isotretinoin therapy is effective in acne vulgaris, gram-negative folliculitis (Plewig et al., 1982), acne fulminans, acne conglobata, dissecting perifolliculitis of the scalp (perifolliculitis capitis abscedens et suffodiens) (Bjellerup and Wallengren, 1990), and acne rosacea (Plewig et al., 1982). Hidradenitis suppurativa may also respond to treatment with isotretinoin, but the response may be partial even with prolonged therapy at 2 mg/kg/d. In this disorder, isotretinoin can be helpful before surgical excision by reducing and delineating the extent of diseased tissue to be removed. Low-dose isotretinoin can produce a rapid therapeutic response in gram-negative folliculitis. This response is not considered to be a direct antibacterial effect, but rather a secondary effect of alterations in the microenvironment.

Inhibition of sebum production with alterations in the chemistry of the skin-surface lipid film may represent a key mechanism of action of isotretinoin in producing clinical improvement in acne (Farrell et al., 1980). At peak levels of sebum suppression, the relative percentage of the skin-surface lipid film, comprising wax esters and squalene, which are derived from the sebaceous glands, is reduced, and the percentage of cholesterol and cholesterol esters is increased. Isotretinoin is the most effective known inhibitor of sebum production, being superior to estrogen and X-irradiation. Inhibition of quantitative sebum production (or the sebum secretion rate) is almost maximal by the fourth week of treatment with isotretinoin, and thus usually occurs prior to clinical improvement. The inhibition is dose-dependent, and doses of 0.5 to 1.0 mg/kg/d lead to an 80 to 90% inhibition after 12 to 16 weeks of therapy (Goldstein et al., 1982). After treatment is stopped, quantitative sebum production returns toward pretreatment levels, but long-term follow-up, from 20 to 99 weeks after treatment, shows an overall persistent 38% (range, 0–80%) inhibition (Strauss et al., 1980). These data suggest that in some patients, continued partial inhibition of sebaceous glands may contribute to the prolonged remission of acne. The histologic changes during and after isotretinoin therapy parallel and reflect the inhibition of sebum production. The sebaceous glands virtually disappear during treatment with isotretinoin and gradually recover after the treatment is discontinued (Landthaler et al., 1981).

The inhibition of the wax-ester secretion rate by isotretinoin was studied specifically, using a bentonite clay technique (Stewart et al., 1983, 1984). The mean rates of wax-ester secretion were greatly elevated in untreated acne patients, but were suppressed below the normal range (as measured in patients without acne) during isotretinoin therapy. However, the post-treatment secretion rates again rose above the normal range for all dose levels of isotretinoin (0.1 to 1.0 mg/kg/d), indicating that other factors must contribute to the continued healing of acne and to the prolonged remissions observed after isotretinoin therapy is discontinued.

In addition to inhibition of sebum production, the mechanisms by which isotretinoin may act in the treatment of acne include anti-inflammatory effects, antibacterial effects, inhibitory effects on microbial enzyme activity, and desquamative effects on poral occlusion (Camisa et al., 1982; King et al., 1982; Leyden and McGinley, 1982). Isotretinoin markedly reduces the number of *Propionibacterium acnes* on the skin surface. This reflects decreased follicular colonization by *P. acnes* secondary to the isotretinoin-induced decrease in sebaceous secretion, and not an antibacterial effect of isotretinoin. The anti-inflammatory effect may be a function of nearly complete inhibition of monocyte and neutrophil chemotaxis during isotretinoin therapy (Norris et al., 1987).

Initial studies suggested that isotretinoin does not act as an antiandrogen since no change was noted in serum androgen levels (Matsuoka et al., 1989) or gonadotropins during isotretinoin therapy, nor were there signs of feminization in treated males. Furthermore, in studies of the hamster flank organ, androgen-sensitive components aside from the sebaceous gland did not involute during treatment with isotretinoin (Gomez, 1981). In contrast to these results, one recent report documents a significant reduction in serum testosterone and urinary steroid metabolites and a change in the ratio of 5-α and 5-β metabolites during isotretinoin therapy, suggesting that 5-α-reductase activity may be sensitive to isotretinoin (Rademaker et al., 1991). In addition, isotretinoin reduced serum levels of dihydrotestosterone and 3α-androstanediol glucuronide, possibly as a result of the reduction in size of sebaceous glands, which may release tissue-derived androgens into the circulation (Lookingbill et al., 1988).

Psoriasis

Unlike acne, in which a single 4- or 5-month course of therapy with isotretinoin can lead to prolonged remission, the treatment of psoriasis with retinoids usually requires their long-term administration, since relapses eventually occur in virtually all patients if therapy is dis-

continued (Fig. 2). Because of the prolonged administration of retinoids, psoriasis patients are at greater potential risk of developing chronic retinoid toxicity than are acne patients. Etretinate at a dosage of 0.5 to 1.0 mg/kg/d is superior to isotretinoin in the treatment of psoriasis vulgaris (Ward et al., 1983; Ehmann and Voorhees, 1982; Mahrle et al., 1982; Goerz and Orfanos, 1978). For long-term therapy, a dosage of about 0.5 mg/kg/d is preferable to higher doses in terms of minimizing acute and chronic toxicities. Approximately 15 to 25% of patients fail to have a satisfactory response to etretinate, in some cases because of a worsening of the disease after therapy is begun, or because of the development of dose-limiting, unacceptable toxicity (Lowe, 1991).

Etretinate is of particular value in the treatment of pustular and erythrodermic psoriasis, the most severe forms of psoriasis. These treatment-resistant variants of the disease often require therapy with potent agents, such as methotrexate or cyclosporine. Pustular psoriasis, palmoplantar pustulosis, and related diseases (Slawsky and Libow, 1990; van Dooren-Greebe et al., 1989), are characterized by prominent neutrophilic infiltration into the epidermis, with the development of clinically apparent pustules. Neutrophils may infiltrate the epidermis by means of chemotactic factors present in the stratum corneum. The action of retinoids on desmosomes leads to the shedding of stratum corneum cells containing these chemotactic factors, and may represent one mechanism by which etretinate is effective in pustular psoriasis. In addition to possibly decreasing the expression of chemotactic factors in the skin, retinoids may directly modify neutrophil function. Isotretinoin is, in addition to etretinate, effective in pustular psoriasis, providing a rapidly excreted, alternative retinoid for women of childbearing potential (Sofen et al., 1984).

The combination of retinoids with other effective therapies for psoriasis has been used to increase therapeutic effectiveness and minimize toxicity. Etretinate at lower doses has been used in combination with photochemotherapy (PUVA), anthralin, ultraviolet radiation (UVB at 280 to 320 nm), and topical corticosteroids (Fritsch et al., 1978; Grupper and Berretti, 1981; Lauharanta et al., 1981a; Orfanos et al., 1979; Van der Rhee and Polano, 1981; Orfanos and Runne, 1976; Honigsmann and Wolff, 1989). Recent reports of retinoids in psoriasis have focused on the use of acitretin, the free-acid metabolite of etretinate, because of its shorter half-life and supposedly smaller potential for post-treatment teratogenicity. Acitretin, at doses of 40 to 50 mg/d, either as monotherapy or in combination with other agents, is comparable to etretinate in efficacy and toxicity (Gollnick, 1991). However, the pharmacokinetics of these drugs are not completely understood. Etretinate has recently been detected in the serum of acitretin-treated patients, raising concerns about prolonged retinoid retention and teratogenic risk (Wiegand et al., 1991).

Several small, uncontrolled studies have indicated that most patients with psoriatic arthritis improve when treated with etretinate, exhibiting fewer tender joints and decreased duration of morning stiffness (Stollenwerk et al., 1981; Klinkhoff et al., 1989). The etretinate-induced improvement allowed patients to decrease or discontinue their use of nonsteroidal anti-inflammatory agents.

Etretinate markedly augmented the response of psoriasis to photochemotherapy with oral methoxsalen and long-wave ultraviolet radiation (UVA, 320 to 400 nm) or PUVA (Fritsch et al., 1978; Grupper and Berretti, 1981; Lauharanta et al., 1981a). The regimen in which a retinoid is combined with photochemotherapy has been termed RePUVA. In many studies of RePUVA, etretinate has been given for 1 to 4 weeks, followed by the addition of PUVA. The combined treatment considerably decreased the total amount of UVA needed for clearing psoriasis, and accelerated the response of the disease to PUVA. Moreover, the combined treatment was effective in patients in whom PUVA had previously failed. The RePUVA approach produced longer remissions than those induced by PUVA. Fewer side effects from etretinate were seen during RePUVA than with etretinate used alone, owing to the lower doses employed.

The RePUVA approach, using isotretinoin at a dose of 1 mg/kg/d, was compared prospectively to RePUVA with etretinate (Honigsmann and Wolff, 1983). The retinoids were given alone for 5 days before adding PUVA, and were discontinued once the patients' psoriasis had cleared completely, at which time the patients were placed on PUVA maintenance. Even though etretinate is superior to isotretinoin when used alone in the treatment of psoriasis vulgaris, no significant difference between the two treatment regimens was observed with regard to the duration of treatment needed for clearing of the disease, the number of UVA exposures required, or the cumulative UVA dose. Because isotretinoin is rapidly cleared from the body, with a much shorter risk of teratogenicity after discontinuation of therapy, the use of isotretinoin–PUVA has a definite advantage over etretinate–PUVA in women with childbearing potential.

Once psoriasis has cleared or markedly improved with etretinate, the subsequent post-treatment clinical course is variable. Some patients have prolonged remissions without maintenance therapy. In other patients the therapeutic effect can often be maintained with conventional topical therapy and ultraviolet radiation, with or without low-dose etretinate (about 25 mg/d). However, relapses may occur even if etretinate therapy is maintained. Chronic maintenance therapy with etretinate may be necessary for patients with pustular psoriasis or erythrodermic psoriasis, and for those patients with severe psoriasis vulgaris who have proved to be resistant or intolerant of other treatments and who regularly demonstrate relapse on withdrawal of etretinate.

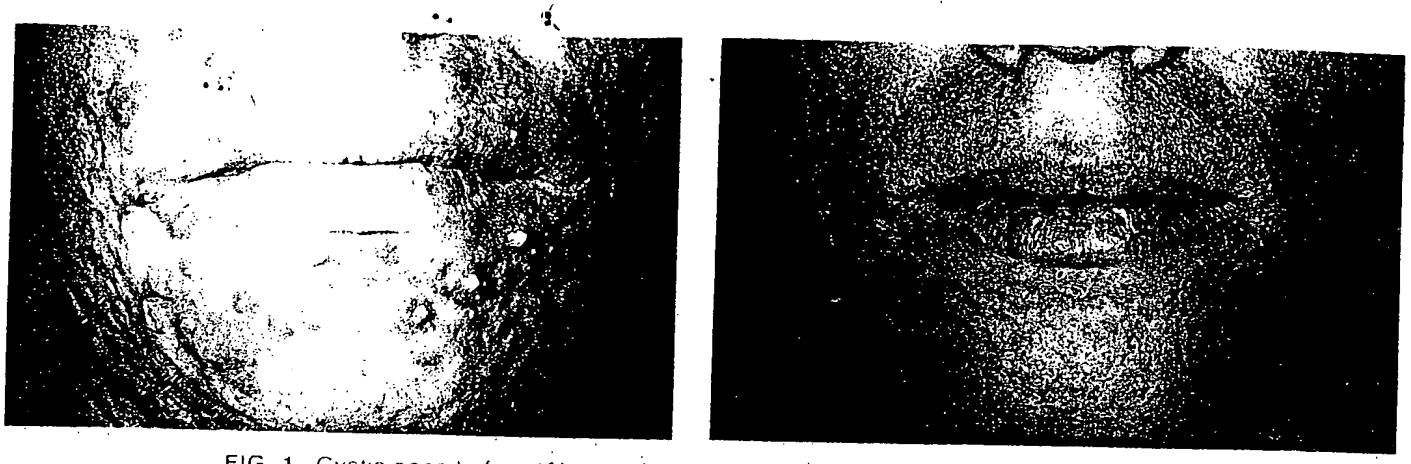


FIG. 1. Cystic acne before (A) and after (B) 4 months of treatment with isotretinoin.

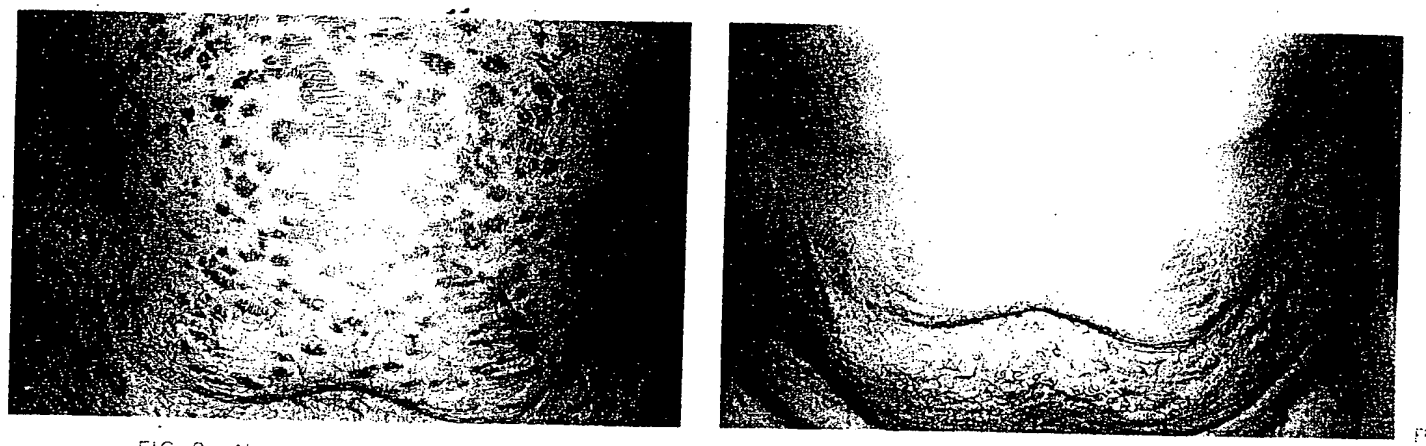


FIG. 2. Widespread chronic psoriasis before (A) and after (B) 4 months of treatment with etretinate.

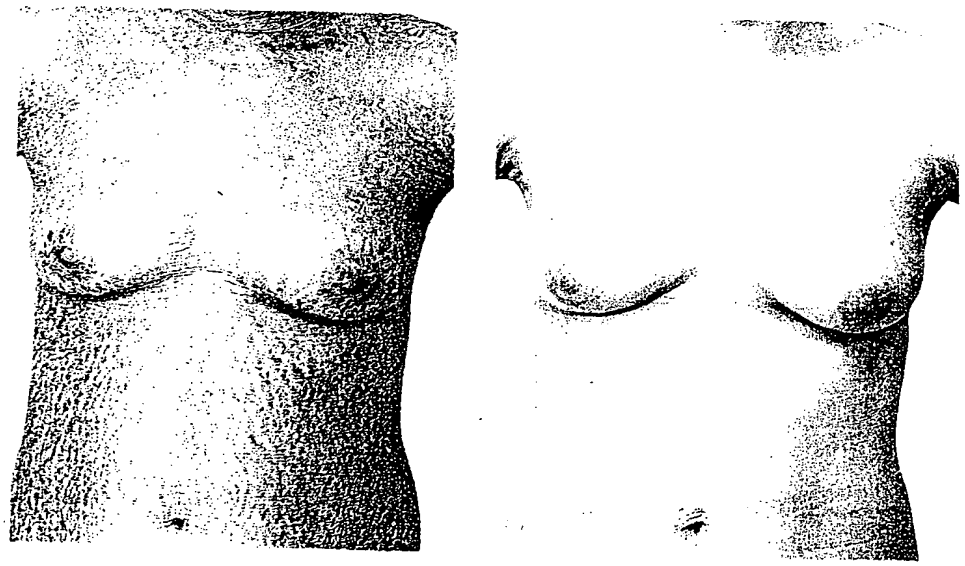
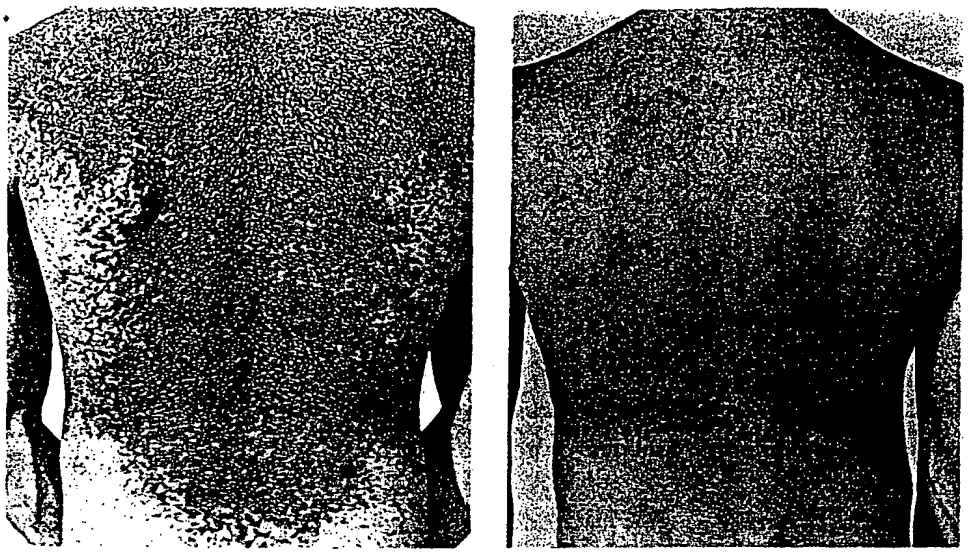
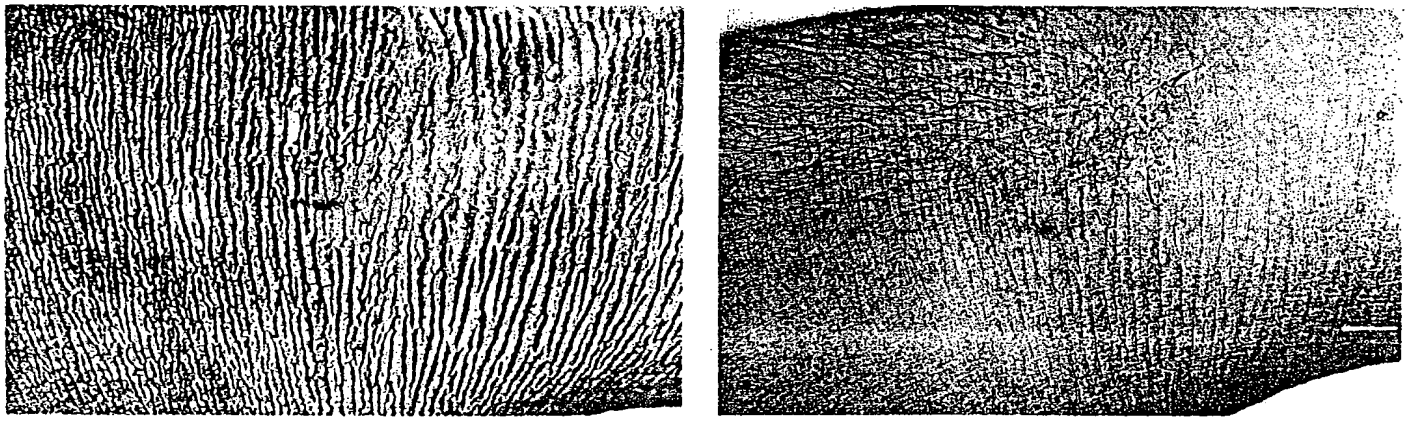


FIG. 3. Generalized lamellar ichthyosis before (A) and after (B) treatment with etretinate.



A,B

FIG. 4. Severe Darier's disease before (A) and after (B) 4 months of treatment with etretinate.



A

FIG. 5. Epidermolytic hyperkeratosis (antecubital fossa) before (A) and after (B) 2 months of treatment with etretinate

A

FIG. 6. (A) Multiple basal-cell carcinomas prior to treatment (B) After 8 years of therapy with isotretinoin, no new tumors have appeared. Pre-existing lesions were treated surgically. [From *Dermatologica* 1987;175(Suppl 1):138–144, with permission of S. Karger AG, Basel.]

Initial dosage recommendations for the treatment of erythrodermic psoriasis with etretinate have been 25 to 35 mg/d, increasing to 50 to 60 mg/d within 2 to 4 weeks (Orfanos et al., 1981). Pustular psoriasis may require doses of 75 mg/d, and chronic psoriasis vulgaris may be treated with 50 mg/d in combination with other active agents. Worsening of psoriasis may occur during the induction of etretinate therapy. This initial worsening is different from the retinoid-related dermatitis produced by high-dose etretinate, which can mimic psoriasis and which resolves with a reduction of dosage or discontinuation of therapy.

Cutaneous Disorders of Keratinization

In 1976, isotretinoin was shown to be effective in previously recalcitrant cases of disorders of keratinization, such as Darier's disease and pityriasis rubra pilaris (Peck and Yoder, 1976; Peck et al., 1978). Since then, many reports have indicated that these and other disorders of keratinization respond to isotretinoin, etretinate, and newer retinoids (Orfanos et al., 1987). In contrast to the results in acne, for which isotretinoin is more effective than etretinate, both retinoids gave comparable responses in lamellar ichthyosis (Fig. 3), Darier's disease (Fig. 4), non-bullous congenital ichthyosiform erythroderma, and pityriasis rubra pilaris (Peck et al., 1981; Lowhagen et al., 1982; Goldsmith et al., 1982; Borok and Lowe, 1990). Etretinate was superior to isotretinoin in the treatment of psoriasis, epidermolytic hyperkeratosis (Fig. 5), keratoderma palmaris et plantaris, X-linked ichthyosis, ichthyosis vulgaris, erythrokeratodermia variabilis, Papillon-Lefevre syndrome, Kyrle's disease, and lichen planus (Burge, 1989). Harlequin fetus, a frequently lethal congenital ichthyosis, responds well to etretinate (Lawlor and Peiris, 1985; Ward and Jones, 1989) and to isotretinoin (Roberts, 1989), with long-term survival and the resolution of ectropion and eclabion. Recently, acitretin was demonstrated to be as effective as etretinate in treating many of these disorders (Blanchet-Bardon et al., 1991; Laurberg et al., 1991). Temarotene (Ro 15-0778), an arotinoid lacking a polar end group, was effective in clearing lichen planus with less toxicity than currently available retinoids (Bollag and Ott, 1989).

Patients with the dry, brown, hyperkeratotic type of Darier's disease respond better to etretinate and may have more prolonged remissions than those with the red, inflamed, infected variety of Darier's disease who also have marked intertriginous involvement. The latter patients are much more difficult to treat, and relapse very quickly after treatment is discontinued. In one report, isomorphic reactions were noted to occur in one-third of patients with Darier's disease treated with etretinate (Lowhagen et al., 1982).

In a report of 45 patients with pityriasis rubra pilaris of varying duration prior to therapy, long-term remissions were noted after the discontinuation of isotretinoin (Goldsmith et al., 1982). Although most cases of adult-onset pityriasis rubra pilaris clear spontaneously within 3 years, this finding could indicate either that isotretinoin therapy induced or accelerated a spontaneous remission or was merely coincidental with it. In patients who did not have a complete remission after a course of therapy, new areas of involvement did not occur, and recurrent disease did not reach the pretreatment degree of disease severity. This contrasts with what has been observed in Darier's disease after stopping treatment with isotretinoin. Intermittent courses of therapy with prolonged treatment-free intervals may be effectively used in patients such as these. However, not all patients with pityriasis rubra pilaris respond in this manner. For instance, two patients with chronic pityriasis rubra pilaris, characterized by childhood onset, a myriad of follicular papules, and a disease duration of longer than 10 years responded very dramatically to treatment initially with isotretinoin and subsequently with etretinate. These patients relapsed dramatically and completely after each 4- to 6-month course of therapy over a more than 8-year period of retinoid therapy (Peck et al., 1981).

Patients with lamellar ichthyosis treated with retinoids had a reduction in scaling, an increased heat tolerance and ability to sweat, and improvement in their ectropion. Clearing in these patients is usually not complete, and may be greater in the summer than in the winter, when their disease is typically more severe.

Since disorders of keratinization may require long-term therapy with retinoids, the safety of chronic retinoid administration must be addressed. Of particular concern is bone toxicity. In children, premature closure of epiphyses (Milstone et al., 1982) and fractures (Tamayo and Ruiz-Maldonado, 1981) can rarely occur, as can changes in the axial skeleton resembling diffuse idiopathic skeletal hyperostosis in both children and adults (Pittsley and Yoder, 1983; Gerber et al., 1984; Ellis et al., 1984).

Unusual responses to retinoid therapy have been observed in patients with cutaneous disorders of keratinization (Fritsch, 1981). It was observed that palmoplantar blistering could be enhanced during the etretinate therapy of keratoderma palmaris et plantaris (epidermolytic type), epidermolytic hyperkeratosis, and pachyonychia congenita. Patients with Hailey-Hailey disease and atopic dermatitis have worsened with retinoids, particularly at higher doses.

Miscellaneous Diseases

Both discoid and subacute cutaneous lupus erythematosus respond well to retinoid therapy (isotretinoin, etre-

tinate, and acitretin), with either a total clearance or marked reduction of skin lesions. Retinoids have been used alone or in combination with other therapies, such as antimalarial drugs or systemic corticosteroids, and have been effective against lupus even in patients who had proved resistant to these other modalities. Since most patients with lupus erythematosus are women of childbearing potential, isotretinoin and acitretin are preferable to etretinate because of their shorter elimination half-lives, which limit the risk of post-treatment teratogenicity. (Ruzicka et al., 1988; Shornick et al., 1991).

Cancer

The synthetic retinoids, isotretinoin and etretinate, have been used in the treatment and prevention of a variety of cutaneous malignancies. These include chronic sunlight damage (basal-cell carcinoma, actinic keratosis) (Moriarity et al., 1982), nevoid basal cell carcinoma syndrome (Peck et al., 1978; Peck et al., 1982a; Peck et al., 1988; Goldberg et al., 1989; Sanchez-Conejo-Mir and Camacho, 1989; Hodak et al., 1987), xeroderma pigmentosum (basal-cell carcinoma, squamous-cell carcinoma, keratoacanthoma, actinic keratosis) (Kraemer et al., 1988; Braun-Falco et al., 1982; Berth-Jones and Graham-Brown, 1990), multiple keratoacanthomas (Haydey et al., 1980; Berretti et al., 1981; Wright et al., 1988; Street et al., 1990), porokeratosis of Mibelli with malignant degeneration (squamous-cell carcinoma, Bowen's disease) (Schnitzler and Verret, 1981), epidermodysplasia verruciformis (Lutzner and Blanchet-Bardon, 1980), Cowden's disease (Lazar and Lazar, 1986), Muir-Torre syndrome (Spielvogel et al., 1985), oral leukoplakia (Koch, 1981; Hong et al., 1986), cutaneous metastases of malignant melanoma (Levine and Meyskens, 1980), and cutaneous T-cell lymphoma (mycosis fungoides and Sézary's syndrome) (Souteyrand et al., 1981; Kessler et al., 1983; Lippman and Meyskens, 1989). Synthetic retinoids do not usually cure cutaneous tumors, but do produce variable degrees of partial regression when given at high dosage. The mechanism of action by which they accomplish this is not clear. Induction of inflammation by high-dose isotretinoin is not a necessary prerequisite for the regression of basal-cell carcinoma, since both inflamed and non-inflamed tumors may undergo regression (Peck et al., 1978, 1982a, 1988).

If the clinical goal is changed from chemotherapy to chemoprevention, synthetic retinoids can have dramatic effects in preventing the formation of new skin tumors. The benefit persists only as long as therapy is maintained, particularly in patients with precancerous genodermatoses (nevoid basal cell carcinoma syndrome, xeroderma pigmentosum) (Kraemer et al., 1988; Peck et al., 1982a, 1988).

Pilot Study of Isotretinoin for Basal Cell Carcinoma

Oral isotretinoin was first used in patients with multiple basal cell carcinomas in a two-stage trial. In the first treatment stage, high doses of isotretinoin were employed as chemotherapy. The second stage used lower doses for chemoprevention of recurrent disease (Peck et al., 1982a).

Chemotherapy

Twelve patients with a total of 270 basal cell carcinomas due to chronic sunlight exposure, X-irradiation, arsenical insecticide exposure, and nevoid basal-cell carcinoma syndrome received an average maximum dosage of 4.5 mg/kg/d of oral isotretinoin. The mean duration of treatment at these dosages was approximately 8 months. In this series there was complete clinical remission of 43 tumors (16%). Of these 43 tumors, 35 underwent biopsy: 21 specimens were free of tumor and 14 were not. Thus, approximately 10% of the tumors underwent complete clinical and histologic remission. A marked individual variation was evident in the therapeutic response. For example, one patient had 15 of 37 lesions undergo complete clinical regression, whereas another had none of his 8 lesions completely disappear clinically. This variation in therapeutic response did not seem to be a function of dosage, tumor location, tumor etiology, duration of therapy, or the age or sex of the patient.

With the high-dose isotretinoin, inflammation developed in the tumors of some patients but not in the adjacent skin, which was clinically free of tumor. After treatment was discontinued and the inflammatory reaction subsided, an objective reduction in tumor mass was noted in those tumors in which an inflammatory reaction had occurred. However, other patients demonstrated clinical involution of their tumors without signs of inflammation, indicating that tumor regression need not be mediated by inflammation. In some patients, therapy with isotretinoin caused previously undetected lesions to become inflamed, resembling what is observed with topical 5-fluorouracil in the treatment of solar keratoses. Smaller tumors responded better to high-dose isotretinoin; 23% of 3- to 5-mm tumors exhibited a complete response, in contrast to only 7% of tumors 10 mm or larger. Lower doses (0.5 to 1.5 mg/kg/d) of isotretinoin were ineffective as chemotherapy, producing no complete regressions and only partial regression in one tumor. No tumors became inflamed at these lower dosages. Because these results compared unfavorably with the results of standard therapy, the use of oral isotretinoin as a chemotherapeutic agent for basal cell carcinoma was abandoned.

Chemoprophylaxis

The lower-dose phase of this pilot study included only 3 of the 12 patients involved in the chemotherapy phase. During the first 34 months of the chemoprophylaxis phase, an average dosage of 1.5 mg/kg/d of oral isotretinoin was used. Subsequently, the dosage was lowered to 40 mg/d (0.5 mg/kg/d). While each of the three previously studied patients had differing percentages of a complete response (3%, 12%, 45%) to isotretinoin during chemotherapy, none developed new tumors within the next 2 to 8 years. One of these patients, who had the nevoid basal cell carcinoma syndrome, had developed 25 new tumors per year for many years prior to therapy, but developed only one tumor per year during his 7-year treatment period with isotretinoin. After isotretinoin therapy was stopped because of skeletal toxicity, this patient developed 29 tumors in the first post-treatment year. The rapid post-treatment development of new tumors in this patient was similar to that observed in those patients who withdrew from the trial because they could not tolerate the mucocutaneous toxicities of isotretinoin at high dosage. In these patients, pre-existing lesions enlarged and new tumors began to appear at varying intervals after therapy ceased. This indicated that the chemopreventive effects of retinoids would require chronic maintenance therapy. Another patient on long-term chemoprophylaxis, whose skin cancers were secondary to arsenical insecticide exposure, averaged 5 tumors per year for 6 years prior to being treated with isotretinoin, but no new tumors during the 8 years of isotretinoin therapy (Fig. 6). However, this patient is unique in that he has developed only one new tumor in the first 4 years after the discontinuation of therapy (Peck, 1987).

Toxicities

The toxicities observed in the trial of isotretinoin for basal cell carcinoma were dose-dependent and were more frequent and severe during the initial high-dose, chemotherapeutic phase of this study. The common (at least 50% of patients) toxicities observed at high-doses of isotretinoin included mucocutaneous toxicity (cheilitis, facial dermatitis, xerosis with itching, dryness of the nasal mucosa with minor nosebleeds, fragility of the stratum corneum), systemic toxicity (arthralgias/myalgias, fatigue) and laboratory abnormalities {hyperlipidemia, abnormal liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST)]}. One chronic toxicity of high-dose isotretinoin was observed in these patients. Radiographic abnormalities of the spine, retinoid hyperostosis (calcification of the anterior spinal ligament with osteophyte and bony-bridge formation) were found in four of eight patients with multiple basal cell carcinomas treated with high-dose isotretinoin (2 mg/kg/d or more) for a minimum of 2 years (Gerber et al., 1984).

Prevention of Skin Cancer in Xeroderma Pigmentosum with Isotretinoin[1]

Xeroderma pigmentosum is an extremely rare, autosomal recessive disorder with an incidence of about one per million, and is characterized by sun sensitivity and a deficiency in the repair of ultraviolet-damaged DNA (Kraemer et al., 1987). Skin cancers develop in this disease at a frequency greater than 1,000 times that in the general population. Thus, patients with xeroderma pigmentosum are ideal candidates for studies of cancer prevention in humans.

Subsequent to the initial reports described above (Peck et al., 1978 and 1982a), a controlled prospective study was conducted to determine whether high-dose oral isotretinoin (2 mg/kg body weight/d), given for 2 years, was effective in preventing the development of new skin cancers in patients with xeroderma pigmentosum (Kraemer et al., 1988). Control was achieved by comparing the frequency of tumors in each patient during treatment with their frequency during the 2-year period before treatment and during a 1-year period after treatment.

Seven patients with xeroderma pigmentosum were enrolled in the protocol; five completed the study as outlined, without dose modification (Fig. 7). Two patients were unable to complete the protocol because of persistent laboratory-test abnormalities (hyperlipidemia, abnormal liver function tests) during treatment. The total number of tumors in the five patients who completed the study decreased from 121 (mean, 24; range, 8 to 43) in the 2 years before treatment to 25 (mean, 5; range, 3 to 9) in the 2 years of therapy, with a mean reduction in the tumor rate of 63% ($p = 0.019$) (Fig. 7). In four of the five patients there was a reduction in the number of tumors during treatment as compared with that before treatment. In these four patients, the onset of improvement was noted within 2 months of the start of treatment, and the reduction in tumor frequency persisted so that during the 2-year treatment interval there was a 70 to 93% decrease in the frequency of skin cancers ($p = 0.006$). The one patient who did not have a decrease in tumor frequency during treatment had 8 histologically documented tumors in the 2 years before treatment and 9 during the 2 years of treatment.

During the post-treatment observation period, which lasted a minimum of 9 months, an increase in the fre-

[1] Portions of this section are reprinted, with permission, from Kraemer et al., *N Engl J Med* 1988;318:1633–1636.

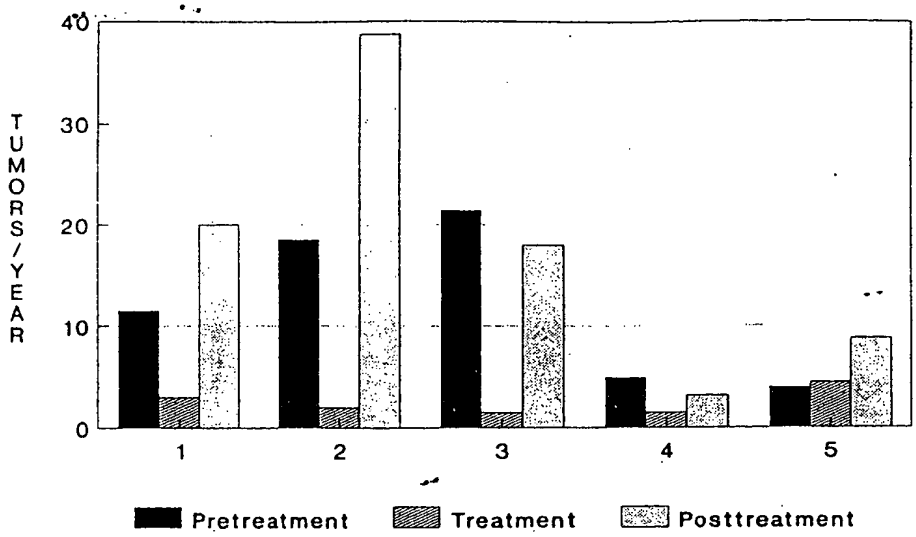


FIG. 7. Inhibition of tumor formation is observed during treatment with isotretinoin (2 mg/kg/day) in four of five patients with xeroderma pigmentosum. Tumor incidence increased in all five patients after cessation of therapy. Durations of pretreatment and treatment periods were each 2 years; post-treatment follow-up periods ranged from 9 to 14 months.

quency of skin cancers was observed in all five of the patients who completed the study. This increase usually became apparent within 3 months after stopping treatment. A mean 8.5-fold increase in the annual rate of tumor occurrence (range, 2- to 19-fold) was observed in the post-treatment period as compared with the treatment period ($p = 0.007$).

Post-treatment tumor rates were at least as high as those in the pretreatment interval. Three patients had about a twofold increase in their tumor-removal rate after treatment as compared with that before treatment. Thus, the treatment may have suppressed an acceleration in tumor formation in these patients. Alternatively, there may have been a temporary rebound in tumor incidence after the withdrawal of therapy.

The best response to therapy was observed in those patients who had had the highest frequency of tumors before treatment. The decrease in tumor frequency during treatment, as compared to the tumor incidence observed during the pretreatment period, and the increase in post-treatment tumor frequency compared to the 2-year treatment period seen in four of five patients was significant ($p = 0.045$ by the nonparametric generalized sign test).

Side effects of oral isotretinoin at 2 mg/kg/d included moderate to severe mucocutaneous toxicities (cheilitis, xerosis, conjunctivitis) and systemic toxicities (arthralgias, hypertriglyceridemia, abnormal liver function tests) in the majority of patients. In addition, poor wound healing, secondary infection with *Staphylococcus aureus*, and pyogenic granulomas were noted. Two patients developed skeletal toxicity resembling diffuse idiopathic skeletal hyperostosis, including vertebral bony bridging and extraspinal involvement, such as calcification of the Achilles tendons and plantar ligaments in the feet. We consider these toxicities to be substantial and excessive for long-term therapy with isotretinoin at 2 mg/kg/d for the prevention of non-melanoma skin cancer. Therefore, we are currently exploring the use of lower doses (0.5 to 1.5 mg/kg/d) of oral isotretinoin for the prevention of skin cancer in the patients who completed the high-dose study.

All seven patients who were enrolled in the high-dose phase of this study were included in the subsequent low-dose trial of isotretinoin for xeroderma pigmentosum (Kraemer et al., 1990). The five patients who completed 2 years of isotretinoin therapy at 2 mg/kg/d without dose modification were included, as were the two patients whose dose had to be lowered due to toxicity. The patients were treated with oral isotretinoin at 0.5 mg/kg/d for 1 year and monitored for the incidence of new tumors and toxicity (Kraemer et al., 1990). In comparison to the interval without treatment, the frequency of skin cancers occurring during the low-dose treatment decreased in most patients. In some patients there was an apparent dose response. Furthermore, mucocutaneous toxicity and laboratory abnormalities were less severe with the low-dose treatment. This study is continuing in an effort to better define the chemopreventive effect and observed toxicities relative to the dose of isotretinoin. Thus far, the minimum effective dosage required to prevent the formation of basal cell carcinomas varies between individual patients and ranges from 0.5 to 1.5 mg/kg/d, or 40 to 120 mg/d. These results are comparable to those obtained in patients with the nevoid basal cell carcinoma syndrome.

This study indicates that high-dose (2 mg/kg/d) isotretinoin is effective in preventing the formation of new skin cancers in patients with xeroderma pigmentosum, and represents the first controlled, prospective study to demonstrate cancer chemoprevention in humans. Withdrawal of therapy resulted in a rapid reversal of the che-

moprophylactic effect, with a significant increase in tumor rates over that in the treatment period. These findings firmly link isotretinoin therapy with tumor suppression. Our data suggest that isotretinoin suppresses tumor promotion or progression, perhaps by preventing the conversion of premalignant to malignant lesions or by arresting the growth of existing malignant lesions that were too small to be identified clinically. The period required for the tumorigenic process to progress from the initial sun-induced injury to the formation of a malignant skin tumor in patients with xeroderma pigmentosum is thought to be several years. In our patients, isotretinoin seemed to act as a switch, turning off the appearance of tumors within 2 months of the onset of therapy and quickly losing effectiveness within 3 months of the cessation of treatment. The speed at which this preventive effect can be switched on and off is probably too rapid to result from alterations in the initial mutational steps in tumor formation, since such alterations would not be expected to prevent tumors for several years.

Multicenter Trial of Low-Dose Isotretinoin for Basal Cell Carcinoma

On the basis of the results of the pilot study described above (Peck et al., 1982a), the chronic administration of low-dose isotretinoin (10 mg/d or 0.14 mg/kg/d) was evaluated for efficacy in the prevention of new basal cell carcinomas in patients with a history of at least two basal cell carcinomas in the 5 years prior to entry into the study (Tangrea et al., 1990; Tangrea et al., 1992a).

The 10 mg daily dose used in this trial was specifically chosen on the basis of patient acceptability, given the drug's chronic dosing schedule and expected side effects, and to avoid possible unmasking of the trial by the characteristic mucocutaneous toxicity of isotretinoin. Isotretinoin at this dose level has been clinically useful, with minimal morbidity, in the treatment of benign dermatoses (Farrell et al., 1980; Jones et al., 1983). This study was a randomized, double-blind, placebo-controlled trial with a sample size of 981 patients, and was coordinated by the National Cancer Institute and conducted at eight clinical centers in the United States. Analyses were made of the cumulative incidence curves (drug vs. placebo) of the first new basal cell carcinoma over the 3-year treatment period and of the multiplicity of basal cell carcinomas in the treatment and placebo groups by comparing tumor rates (defined as the total number of basal cell carcinomas for all visits divided by the number of person-years of follow-up).

In contrast to previous studies of isotretinoin at higher doses, the results of this study indicated no significant difference between the treatment and placebo groups ($p = 0.72$) in the comparison of the 3-year cumulative incidence curves for new basal cell carcinomas. At the end of 3 years of intervention, 327 patients in the isotretinoin group and 327 patients in the placebo group had developed at least one new basal cell carcinoma. Isotretinoin also had no effect on the multiplicity of lesions as measured by the rate of basal cell carcinoma formation. The tumor rate was 0.94 tumors per patient per year in the isotretinoin group and to 0.96 per patient per year in the placebo group ($p = 0.83$). While the cumulative incidence rates and tumor rates were highest in males, older patients, patients with the greatest prior solar damage, and most strongly in patients with the greatest number of basal cell carcinomas in the 5 years prior to entering the study, there was no indication of a protective effect of isotretinoin in any of these subgroups.

Adverse reactions, reported both in the isotretinoin group (76% of patients) and in the placebo group (43% of patients), were mild, and dose modification was considered necessary for fewer than 20% of the reactions. Significant differences in observed side effects were noted between the treatment and placebo groups for mucocutaneous toxicities and hypertriglyceridemia, and in the development and progression of cervical and thoracic hyperostotic vertebral abnormalities. At the end of the 3-year treatment period, the mean total serum triglyceride level was 1.63 mmol/L in the isotretinoin group and 1.45 mmol/L in the placebo group ($p < 0.001$). The mean total serum cholesterol did not differ between the two groups. Upon comparing baseline versus end-of-treatment (3-year) radiographs of the cervical and thoracic spine in a subsample of 269 patients, 40.3% of the isotretinoin-treated patients and 18.5% of those given placebo exhibited a progression of pre-existing hyperostotic vertebral abnormalities ($p < 0.001$), while 8.6% of the isotretinoin-treated patients and 1.5% of those given placebo developed new hyperostotic involvement of previously unaffected vertebral levels ($p = 0.015$) (Tangrea et al., 1992b). These mild to moderate radiographic changes appeared to be a continuation of pre-existing processes rather than a unique or distinct pathologic change, and were not productive of clinical symptoms.

The lack of benefit of isotretinoin observed in this trial differs from the results of previous studies, in all likelihood because of the much lower dose of isotretinoin used. It should be mentioned, however, that even at the low dose of 10 mg/d, isotretinoin produced measurable systemic toxicity. This finding should be weighed in the risk/benefit analysis of the long-term use of isotretinoin, and must be considered in planning future chemopreventive studies with this retinoid. For otherwise healthy patients, such as those in this multicenter trial, it may be difficult to justify testing much higher doses of isotretinoin, owing to the probable production of more toxicity. However, for patients with precancerous genoderma-

toses (nevoid basal cell carcinoma syndrome, xeroderma pigmentosum) who may develop several dozen skin cancers per year, the potential benefit of moderately higher doses of isotretinoin may outweigh the expected detriment of increased toxicity.

Other Studies

Isotretinoin has been reported to cause the regression of squamous cell carcinoma. Complete and partial regressions occurred in four patients with large, recurrent or metastatic squamous cell carcinomas treated with oral isotretinoin at 1.0 mg/kg/d (Lippman and Meyskens, 1987). The authors suggest that retinoid activity correlates directly with the degree of differentiation of transformed cells, and that the well-differentiated nature of squamous cell carcinomas may therefore have been a factor in the excellent therapeutic responses observed. Isotretinoin, given as a chemopreventive agent, was also found to reduce the rate of second primary squamous cell carcinomas among patients with previously treated head and neck cancers (Hong et al., 1990). Although the chemoprevention of potentially lethal cancers was observed, the high dose of isotretinoin used (50 to 100 mg/m^2) resulted in significant participant toxicity, with 33% of the patients dropping out over the 12-month treatment period.

Recent clinical trials have identified clinical responses to combination therapy with retinoids and cytokines. The chemotherapeutic effect of isotretinoin on advanced squamous cell carcinoma of the skin was enhanced by the addition of interferon-α_{2a}. Seven of 28 assessable patients had complete responses. Although dose reductions were required in the majority of patients, the toxicities of the two treatment agents were reversible and nonoverlapping (Lippman et al., 1992). Similarly, the retinoid-induced differentiation of HL-60 (acute promyelocytic leukemia) cells *in vitro* is enhanced by a variety of cytokines, including interferons, tumor necrosis factor-α (TNF-α), granulocyte colony stimulating factor (GCSF), interleukin-1α and interleukin-4 (Bollag, 1991; Bollag and Peck, 1991). Limited benefit was observed, however, when etretinate and interferon-α were combined as therapy for advanced melanoma (Rustin et al., 1988).

Several reports have described beneficial responses to synthetic retinoids either alone or in combination with other chemotherapeutic agents, including interferon-α (Zachariae and Thestrup-Pedersen, 1990) or PUVA, in the treatment of cutaneous T-cell lymphoma (Souteyrand et al., 1981; Lippman and Meyskens, 1989). When synthetic retinoids were used alone, tumors and plaques underwent a marked partial clinical regression. Relapse occurred in most patients after the cessation of therapy, indicating that treatment with synthetic retinoids is not curative. The synthetic retinoids may be active in cutaneous T-cell lymphoma by virtue of direct effects on the function of subsets of T lymphocytes.

Toxicity

Acute Toxicity

The synthetic retinoid toxicities (Table 2) mimic many of the findings in vitamin A intoxication, but are less severe than those seen with the high doses of vitamin A required for clinical efficacy; they primarily involve the skin and mucous membranes.

The acute toxicities of the two synthetic retinoids, isotretinoin and etretinate, overlap but are not identical. Similarly, it appears that new synthetic retinoids will have unique spectra of clinical toxicity as well as efficacy. Under certain circumstances the differences in relative toxicity will influence retinoid selection in diseases in which the therapeutic effects are comparable.

Mucocutaneous Toxicity

The acute mucocutaneous toxicities commonly observed with isotretinoin and etretinate are well tolerated and not life threatening, are dose dependent in incidence and severity, are treatable with bland therapies, and are reversible on dosage reduction or discontinuation of treatment (Table 2). Cheilitis is the most common toxicity, occurring in almost all patients receiving isotretinoin, even at the low end of the clinically useful dose range. Frequent application of unmedicated lip balms is usually sufficient to alleviate the cheilitis. Facial dermatitis, xerosis with pruritus, dryness of the nasal mucosa with minor nosebleeds, and conjunctivitis are the next most commonly observed mucocutaneous toxicities of isotretinoin and etretinate. Isotretinoin produces more drying and chapping of the skin and mucous membranes than does etretinate. Treatment with etretinate, however, leads to more hair loss and palmar and plantar peeling than observed with isotretinoin.

Conjunctivitis

Conjunctivitis, which may interfere with a patient's ability to wear contact lenses during synthetic retinoid therapy, may result from a decrease in the outer lipid layer of the tear film, with subsequent evaporation of the aqueous phase (Ensink and Van Voorst Vader, 1983). Furthermore, *Staphylococcus aureus* has been cultured from the eyelids of patients with isotretinoin-induced blepharoconjunctivitis (Blackman et al., 1979). If artificial tears and topical ophthalmologic antibiotic therapy fail to relieve the conjunctivitis, then ophthalmologic

consultation should be sought. Other ophthalmologic toxicities from retinoids (isotretinoin, etretinate, fenretinide, acitretin) include corneal opacities, decreased night vision with abnormal electroretinograms, transient acute myopia, papilledema secondary to pseudotumor cerebri, cataracts, and teratogenic abnormalities (Gold et al., 1989; Safran et al., 1991; Brown and Grattan, 1989). An inhibition of ocular retinol dehydrogenases with decreased formation of 11-*cis*-retinal may be the mechanism responsible for retinoid-induced night blindness (Law and Rando, 1989). Fenretinide may be more likely than other retinoids to interfere with night vision because it markedly reduces plasma concentrations of retinol and retinol-binding protein (RBP; Peng et al., 1989; Kaiser-Kupfer et al., 1986; Kingston et al., 1986); however, the likelihood of this toxicity developing during fenretinide therapy can be minimized or avoided entirely by scheduling several treatment-free days during each month of therapy (Costa et al., 1989). Rarely, dry eyes and decreased night vision have been reported to persist after the discontinuation of therapy.

Hair Loss

Hair loss is another toxicologic finding in both hypervitaminosis A and synthetic retinoid therapy. It is more severe during treatment with etretinate. The hair loss usually occurs 3 to 8 weeks after beginning etretinate ingestion, and after a minimum total dose of 2 g. It ceases 6 to 8 weeks after the discontinuation of therapy, although rare instances of persistent hair loss have been reported. In the majority of cases the hair loss is a telogen effluvium; occasionally, dystrophic anagen roots are found (Orfanos, 1980; Berth-Jones et al., 1990). A decreased duration of anagen has been suggested as the cause for the increased plucked telogen count noted after 12 weeks of etretinate therapy at 50 mg/d (Berth-Jones et al., 1990).

Systemic Toxicity

Teratogenicity

Isotretinoin and etretinate are potent teratogens, and fetal deformities are the major concern in treating fertile women with oral retinoids. The birth defects characteristically induced by retinoids (retinoic acid embryopathy) include central nervous system abnormalities (hydrocephalus, microcephaly), external ear abnormalities, cardiovascular abnormalities, facial dysmorphia, eye abnormalities (microphthalmia), and thymus gland abnormalities (Lammer et al., 1985; Stern et al., 1984). In some instances these abnormalities may lead to death. Other reported adverse effects include premature births, parathyroid hormone deficiency, and cases of low intelligence quotient (IQ) scores in the absence of obvious central nervous system abnormality. Although etretinate is a more potent teratogen than isotretinoin in animals, more instances of fetal abnormalities have been associated with isotretinoin than with etretinate (Sulik and Alles, 1991). It is possible that isotretinoin is the more potent teratogen in humans. Alternatively, the increased number of fetal abnormalities with isotretinoin may be due to the differences in age (Dai et al., 1992), fertility, and compliance with birth-control techniques in the different groups of patients treated with these retinoids. In one report, 49% of 396 women, most of them under 30 years old, who were exposed to isotretinoin during pregnancy, had conceived either prior to therapy or during the first 3 weeks of therapy (Dai et al., 1992). With regard to their teratogenicity, there is no known safe dose or duration of therapy of the retinoids. In summary, isotretinoin and etretinate are contraindicated in pregnant women and in women of childbearing potential who refuse to use or are unreliable in complying with effective contraception. Current guidelines are that contraception should be used for 1 month prior to isotretinoin therapy, during therapy, and for 1 month afterwards, and that a negative pregnancy test be obtained 2 weeks before initiating therapy on the second or third day of a normal menstrual period. Etretinate is stored in body-fat depots, has a terminal elimination half-life in plasma of about 100 days, and can be detected in serum in trace amounts for as long as 3 years after the end of therapy (DiGiovanna et al., 1989). Retinoic acid embryopathy has developed even when conception occurred after the discontinuation of etretinate (Lammer, 1988). The current recommendation is to avoid pregnancy for at least 2 years after exposure to etretinate. The effects of retinoids on neural-crest cells during the fourth week after fertilization may be responsible for many of the malformations observed in fetuses of women treated with these agents during pregnancy (Sulik and Alles, 1991).

Acitretin is the carboxylic acid metabolite of etretinate. Its spectra of clinical efficacy and toxicity are comparable to those of etretinate (Gollnick, 1991). Initial multiple-dose pharmacokinetic studies indicated that acitretin differs considerably from etretinate in having a terminal elimination half-life in plasma of only 2 days (Wiegand et al., 1991). This rapid clearance after the discontinuation of acitretin therapy would create the major potential clinical advantage of a reduced risk of delayed teratogenicity in comparison with etretinate. However, recent studies demonstrate that etretinate is found in the plasma, most likely as a metabolite, after the administration of acitretin (Wiegand et al., 1991). It has been suggested that ingested ethanol may play a role in enhancing formation of the ethyl ester of acitretin (Lambert et al., 1992). Further research is warranted to determine whether there is indeed an advantage in using this agent.

Arthralgias and Myalgias

Vitamin A toxicity includes pain and tenderness in bones and joints. Arthralgias have been seen in only a minority of patients treated with synthetic retinoids, and the arthralgias disappear after the discontinuation of therapy. In contrast to these retinoid-induced arthralgias, the treatment of psoriatic arthritis with etretinate has led to objective improvement.

Pseudotumor Cerebri

Pseudotumor cerebri has developed rarely during treatment with isotretinoin. If patients receiving isotretinoin develop a persistent headache with visual changes, nausea, and vomiting, the drug should be discontinued promptly and the patient should be examined for papilledema with retinal hemorrhages. In five such cases the patients were also being treated with tetracycline or minocycline, drugs that are known, albeit rarely, to produce increased intracranial hypertension. This finding would suggest caution in combining these therapies.

Laboratory

Hyperlipidemia

Another acute toxicity common to both retinol and the synthetic retinoids has been hypertriglyceridemia, probably due to both an increased synthesis and decreased elimination of blood lipids. The observed elevations of plasma triglycerides and very-low-density lipoprotein (VLDL) levels have been dose dependent and reversible on discontinuation of therapy (Zech et al., 1983; Bershad et al., 1985). Dosages of isotretinoin above 1 mg/kg/d are usually needed to elevate triglyceride levels markedly beyond the normal range. In one report of 20 men with cystic acne of the trunk who were treated with isotretinoin at a maximum dosage of 2 mg/kg/d, the maximum increases from baseline levels were 67% for triglycerides; 56% for VLDL; 22% for LDL; and 16% for cholesterol. The maximum decrease in high-density lipoproteins was 10%, leading to an increased LDL/HDL ratio (Zech et al., 1983). Hypertriglyceridemia has also been observed with etretinate and acitretin (Vahlquist, 1991), particularly in patients with one of the following predisposing factors: obesity, a high alcohol intake, diabetes, and pretreatment hypertriglyceridemia (Orfanos et al., 1987). One patient, who may have had a pre-existing hyperlipoproteinemia, developed eruptive xanthomas while being treated with isotretinoin at a dose of 2.5 mg/kg/d (Dicken and Connolly, 1980). Another patient developed acute hemorrhagic pancreatitis after the plasma triglycerides exceeded 1400 mg/dL (Shalita et al., 1983). These observations have led to the recommendations for obtaining fasting plasma triglyceride levels prior to initiating retinoid therapy, monitoring triglyceride levels monthly for the first 2 months of therapy and then at 2- to 3-month intervals afterward if they are within normal limits, and discontinuing therapy if the triglyceride levels reach 800 mg/dL. Less severe increases may be treated by dose reduction, dietary management (a low-fat, high-complex carbohydrate diet), a reduction in alcohol and tobacco consumption, and increased physical activity. Although the effect of lipid-lowering medications has not been studied, supplementation with fish oils containing eicosapentenoic and docosahexaenoic acids (MaxEPA) has been reported to reduce triglyceride elevations occurring during retinoid therapy (Marsden, 1989). Certainly if patients with pretreatment elevations in their plasma triglyceride level are to be treated with retinoids, their condition must be monitored very closely. With regard to the risk of atherosclerosis during retinoid therapy, the importance of these observations and the effect of managing plasma triglyceride and cholesterol levels during long-term therapy with retinoids remains to be determined.

Liver

As with vitamin A toxicity, the synthetic retinoids can alter tests of liver function. The tests most commonly showing elevated results are those for the transaminases (AST, ALT), but other tests (alkaline phosphatase, lactic dehydrogenase, bilirubin) can occasionally also be abnormal. Elevations of transaminase occur in approximately 15% of retinoid-treated patients, but usually return to normal within 2 to 4 weeks and remain normal even with continued retinoid therapy. However, acute hepatotoxic reactions to etretinate may occur, with associated fever and eosinophilia, possibly indicating a hypersensitivity to this retinoid (Weiss et al., 1984). Case reports have associated the development of cirrhosis with the long-term use of etretinate (Fallon and Boyer, 1990). However, in prospective studies, long-term therapy with etretinate has not led to chronic liver toxicity, as measured by liver function tests and by liver biopsies examined by light and electron microscopy, even in patients with pre-existing liver disease (Roenigk, 1989).

Semen Analyses

Although retinoids at high doses may inhibit spermatogenesis in animals, semen analyses done on patients receiving oral retinoids have not revealed abnormalities. In fact, a return toward normal of abnormally low pretreatment sperm counts in isotretinoin-treated acne patients has been noted (Schill et al., 1981).

Inflammatory Bowel Disease

Synthetic retinoids rarely have been linked with other toxicities, such as inflammatory bowel disease. However, when four patients with Crohn's disease or ulcerative colitis were treated with isotretinoin for cystic acne, only one patient had an exacerbation requiring the discontinuation of isotretinoin. This suggests that when indicated, isotretinoin may be used with caution in the presence of inflammatory bowel disease (Godfrey and James, 1990).

Chronic Toxicity

The most common findings observed in animals and humans during chronic vitamin-A intoxication have been bony changes. Demineralization, thinning of the long bones, cortical hyperostosis, periostosis, premature closure of the epiphyses, and other changes have all been observed (Frame et al., 1974). Several studies have indicated that the synthetic retinoids may be capable of inducing chronic bone toxicities similar to those in chronic hypervitaminosis A (Lawson and McGuire, 1987; Carey et al., 1988; Ellis et al., 1988; Kilcoyne, 1988; White and MacKie, 1989; DiGiovanna et al., 1986; Teelmann, 1981).

In an early report of bone toxicity, evidence of partial closure of the proximal epiphysis of the right tibia, demineralization, and altered bone remodeling occurred in a 10-year-old boy treated with high doses (3.5 mg/kg/d) of oral isotretinoin over $4\frac{1}{2}$ years for epidermolytic hyperkeratosis (Milstone et al., 1982). In another early report, children with epidermolytic hyperkeratosis, lamellar ichthyosis, psoriasis, and other disorders of keratinization were safely treated with etretinate for more than 3 years, but one child developed two traumatic fractures during this therapy (Tamayo and Ruiz-Maldonado, 1981). Radiologically, this patient's long bones were abnormally slender; however, pretreatment X-rays had not been performed. The administration of etretinate did not interfere with the overall growth and development of these children as monitored by sequential height and weight measurements. Subsequent to these early reports, etretinate has been reported to produce ossification of the interosseous membranes of the forearm, premature epiphyseal closure, additional cases of fractures secondary to the thinning of long bones, periosteal thickening, cortical bone resorption, and osteoporosis (White and MacKie, 1989). It is known that in rats, high-dose vitamin A and high-dose etretinate (3 mg/kg/d) may induce fractures and modeling defects of the long bones, with enhanced bone resorption.

Hypervitaminosis A in adult cats causes the formation of confluent exostoses in the cervical spine (Seawright and English, 1967). Similarly, in 50 patients, of whom 37 were receiving etretinate and 13 were receiving isotretinoin for periods beyond 2 years, 9 had osteophytes present at two or three vertebral levels, with calcification of the anterior spinal ligament but without disc-space narrowing (Gerber et al., 1984). While calcification of the anterior spinal ligament can result from degenerative joint disease, involvement of the ligament occurs in association with narrowing of the intervertebral disc space. In the study by Gerber et al. (1984) the absence of disc-space narrowing was used to eliminate degenerative joint disease as a cause of these changes. Eight of 50 patients had bone bridging, in which osteophytes connected two vertebrae. Patients treated for longer than 2 years with isotretinoin at a minimum dose of 1.5 mg/kg/d were considered to be at significant risk for developing vertebral osteophytes, calcification of the anterior spinal ligament, and bony bridging, similar to the findings in idiopathic skeletal hyperostosis and hypervitaminosis A in the adult. In a skin-cancer prevention study involving patients with xeroderma pigmentosum, calcification of the anterior spinal ligament with bony bridging of vertebrae was observed after 2 years of isotretinoin therapy (2 mg/kg/d) (Kraemer et al., 1988). Rarely, ossification of the spinal posterior longitudinal ligament has been reported. Unlike involvement of the anterior longitudinal ligament, this change, if progressive, may lead to the injury of peripheral nerves exiting from the spinal cord, and even to spinal cord compression with signs of partial spastic paraplegia (Pennes et al., 1985; Lawson and McGuire, 1987). Skeletal changes during retinoid therapy may not require many years to occur. In a prospective study, vertebral osteophytes were observed to form within 12 months after the initiation of therapy with isotretinoin at 2.0 mg/kg/d (Shalita et al., 1983; Ellis et al., 1984). Interference with vitamin D metabolism, leading to reduced serum levels of 1,25-dihydroxyvitamin D [1,25-$(OH)_2D_3$] has been suggested as one possible mechanism by which isotretinoin produces these bony abnormalities (Rodland et al., 1992).

The spectra of skeletal toxicity of various retinoids may differ. Etretinate produces spinal toxicities similar to but perhaps less severe than those from isotretinoin (Gerber et al., 1984; White and MacKie, 1989). In a 4-month study employing bone scintigraphy, 3 of 18 acne patients treated with isotretinoin exhibited pathologic uptake of a technetium radiolabel in the knee and sacroiliac joints, while all 15 psoriasis patients treated with etretinate had normal examinations (Torok et al., 1989). Bone scintigraphy is a very sensitive but nonspecific technique that can document pathologic changes in bone before their detection by conventional X-radiography.

The skeletal abnormalities in spontaneous (i.e., unrelated to retinoid therapy) diffuse idiopathic skeletal hyperostosis (DISH) include the calcification of tendons and ligaments in both the spine and extraspinal locations. However, the diagnosis of DISH depends only on

the extent of spinal involvement. Several studies in isotretinoin-treated patients have identified radiographically evident spinal abnormalities that are sufficiently severe to permit a diagnosis of DISH. The increased risk of spinal involvement associated with isotretinoin therapy has been identified in controlled studies (Gerber et al., 1984; Tangrea et al., 1992b).

Involvement of extraspinal sites has also been identified in individual isotretinoin-treated patients, but the relationship between the spinal and extraspinal involvement is not clear. While spinal involvement has also been observed in etretinate-treated patients, controlled studies for this have not been done. However, a controlled study has found a significant increase in the involvement of extraspinal sites in patients treated with etretinate. Extraspinal tendon and ligament calcification was identified as a common toxicity in a study of 38 patients who received long-term (average, 5 years) etretinate therapy at an average dose of 0.8 mg/kg body weight/d (DiGiovanna et al., 1986). While 32 patients (84%) had radiographic evidence of extraspinal involvement, spinal involvement was uncommon. The extraspinal involvement tended to be bilateral and multifocal. The most common areas of involvement were in the feet (plantar ligament, insertion of the Achilles tendon) and in the pelvis. While the radiographic evidence of extraspinal tendon and ligament calcification did not correlate well with the presence of symptoms, those patients with extensive involvement were symptomatic.

The incidence and severity of bone toxicity may depend on the total dose of retinoid received. In acne patients receiving short-term (4 months) therapy with isotretinoin at doses ranging from 1.0 to 2.0 mg/kg/d, only minimal radiographic abnormalities were observed. Small anterior vertebral spurs developed in only 10 of 96 patients. In some instances the spurs were not visible until 7 to 10 months after treatment (Kilcoyne et al., 1986). Skeletal toxicity has been detected even at very low doses of retinoids. In a placebo-controlled skin-cancer prevention trial using isotretinoin at a dose of only 10 mg/d for 3 years, an enlargement of pre-existing vertebral osteophytes was observed in the retinoid-treated group. At this dose level only a few patients developed new osteophytes (Tangrea et al., 1992b).

Factors in the Decision to Use Retinoids

As with other medications, a risk/benefit ratio should be used to determine whether or not to treat a dermatologic patient with synthetic retinoids. The factors that should be considered include:

1. Responsiveness of the disorder to retinoids. The long remissions of cystic acne after short-term isotretinoin treatment are an optimal response. In some disorders of keratinization, improvement may be only modest, with relapse expected after the cessation of therapy.
2. The dose of retinoid required. Diseases requiring higher doses involve a greater risk. The use of retinoids in combination with other treatments, such as RePUVA for psoriasis, may allow dose reduction.
3. The availability of alternative treatments. In many diseases (psoriasis, acne, lamellar ichthyosis), alternative therapies are available. However, in other diseases (severe Darier's disease, epidermolytic hyperkeratosis) synthetic retinoids may represent the only effective treatment.
4. The chronicity of retinoid therapy. Diseases that relapse rapidly following the withdrawal of synthetic retinoids, and therefore require continuous retinoid administration, are associated with an increased risk of chronic skeletal toxicity. In diseases in which retinoids cause remissions, retinoid-free intervals may be used to reduce the risk of chronic toxicity.
5. The severity of the disease. Disease limitations on educational, psychologic, or physical development should be considered. For example, early retinoid treatment of lamellar ichthyosis may prevent the development of ectropion.
6. The age of the patient. Children with disorders of keratinization requiring chronic moderate- to high-dose retinoid therapy are at greatest risk of developing bone toxicity. They are at risk of premature epiphyseal closure and, because of a longer lifetime exposure to the drug, at higher risk for future development of the vertebral changes resembling diffuse idiopathic skeletal hyperostosis.
7. The sex of the patient. Retinoid teratogenicity entails special risks for the female patient of childbearing age. Although isotretinoin is cleared from the body within days, etretinate can be detected in the serum for months or even years after the discontinuation of its use.
8. The presence of other disorders that may be aggravated by retinoids. Renal or hepatic compromise, pre-existing hyperlipidemia, or a family history of hyperlipidemia or premature atherosclerotic cardiovascular disease should be considered in the therapeutic assessment.
9. The concomitant use of other drugs with similar toxicities. Other drugs than the retinoids are also hepatotoxic (methotrexate), elevate serum lipids (estrogens, corticosteroids), or rarely produce benign intracranial hypertension (tetracycline).

A decade of experience with isotretinoin and etretinate in the treatment of dermatologic disease and prevention of cancer has firmly established a role for synthetic retinoids as efficacious therapeutic agents. The recently identified dramatic response of acute promyelocytic leukemia to tretinoin has stimulated renewed interest in the pursuit of new synthetic retinoid derivatives (such as the arotinoids) (Orfanos et al., 1987) and new indications for the retinoids. With the expanding spec-

trum of retinoid-responsive diseases, and the continuing development of new synthetic compounds, the future of the retinoids holds great promise.

REFERENCES

Adamo, S., De Luca, L. M., Akalovsky, I., and Bhat, P. V. (1979): Retinoid-induced adhesion in cultured, transformed mouse fibroblasts. *J. Natl. Cancer Inst.* 62:1473–1478.

Amos, B., and Lotan, R. (1990): Modulation of lysosomal-associated membrane glycoproteins during retinoic acid-induced embryonal carcinoma cell differentiation. *J. Biol. Chem.* 265:19192–19198.

Bauer, E. A., Seltzer, J. L., and Eisen, A. Z. (1982): Inhibition of collagen degradative enzymes by retinoic acid in vitro. *J. Am. Acad. Dermatol.* 6:603–607.

Bedford, P. A., and Knight, S. C. (1989): The effect of retinoids on dendritic cell function. *Clin. Exp. Immunol.* 75:481–486.

Berretti, B., Grupper, C., Edelson, Y., and Bermejo, D. (1981): Aromatic retinoid in the treatment of multiple superficial basal cell carcinoma, arsenic keratosis and keratoacanthoma. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. pp. 397–399. Springer-Verlag, New York.

Bershad, S., Rubinstein, A., Paterniti, J. R., Le, N. A., Poliak, S. C., Heller, B., Ginsberg, H. N., Fleischmajer, R., and Brown, W. V. (1985): Changes in plasma lipids and lipoproteins during isotretinoin therapy for acne. *N. Engl. J. Med.* 313:981–985.

Berth-Jones, J., Shuttleworth, D., and Hutchinson, P. E. (1990): A study of etretinate alopecia. *Br. J. Dermatol.* 122:751–755.

Berth-Jones, J., and Graham-Brown, R. A. C. (1990): Xeroderma pigmentosum variant: response to etretinate. *Br. J. Dermatol.* 122:559–561.

Bjellerup, M., and Wallengren, J. (1990): Familial perifolliculitis capitis abscedens et suffodiens in two brothers successfully treated with isotretinoin. *J. Am. Acad. Dermatol.* 23:752–753.

Blackman, H. J., Peck, G. L., Olsen, T. G., and Bergsma, D. R. (1979): Blepharoconjunctivitis: a side effect of 13-*cis*-retinoic acid therapy for dermatologic diseases. *Ophthalmology* 86:753–759.

Blanchet-Bardon, C., Nazzaro, V., Rognin, C., Geiger, J.-M. and Puissant, A. (1991): Acitretin in the treatment of severe disorders of keratinization. Results of an open study. *J. Am. Acad. Dermatol.* 24:982–986.

Blumenberg, M., Connolly, D. M., and Freedberg, I. M. (1992): Regulation of keratin gene expression: the role of the nuclear receptors for retinoic acid, thyroid hormone, and vitamin D_3. *J. Invest. Dermatol.* 98:42S–49S.

Bollag, W. (1971): Therapy of chemically induced skin tumors of mice with vitamin A palmitate and vitamin A acid. *Experientia* 27:90–92.

Bollag, W. (1991): Retinoids and interferon: a new promising combination? *Br. J. Haematol.* 79[Suppl 1]:87–91.

Bollag, W., and Ott, F. (1989): Treatment of lichen planus with temarotene. *Lancet* 2:974.

Bollag, W., and Peck, R. (1991): Modulation of growth and differentiation by retinoids and cytokines. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 127–138. Karger, Basel, New York.

Borok, M., and Lowe, N. J. (1990): Pityriasis rubra pilaris. Further observations of systemic retinoid therapy. *J. Am. Acad. Dermatol.* 22:792–795.

Braun-Falco, O., Galosi, A., Dorn, M., and Plewig, G. (1982): Tumor prevention in xeroderma pigmentosum using aromatic retinoid (Ro 10-9359). *Hautarzt* 33:445–448.

Breitman, T. R., Selonick, S. E., and Collins, S. J. (1980): Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc. Natl. Acad. Sci. U.S.A.* 77:2936–2940.

Brinckerhoff, C. E., McMillan, R. M., Dayer, J. M., and Harris, E. D., Jr. (1980): Inhibition by retinoic acid of collagenase production in rheumatoid synovial cells. *N. Engl. J. Med.* 303:432–436.

Brown, R. D., and Grattan, C. E. H. (1989): Visual toxicity of synthetic retinoids. *Br. J. Ophthalmol.* 73:286–288.

Burge, S. M. (1989): Darier's disease and other dyskeratoses: response to retinoids. *Pharmacol. Ther.* 40:75–90.

Cai, D., Ben, T., and De Luca, L. M. (1991): Retinoids induce tissue transglutaminase in NIH-3T3 cells. *Biochem. Biophys. Res. Commun.* 175:1119–1124.

Camisa, C., Eisenstat, B., Ragaz, A., and Weissmann, G. (1982): The effects of retinoids on neutrophil functions *in vitro*. *J. Am. Acad. Dermatol.* 6:620–629.

Carey, B. M., Parkin, G. J. S., Cunliffe, W. J., and Pritlove, J. (1988): Skeletal toxicity with isotretinoin therapy: a clinico-radiological evaluation. *Br. J. Dermatol.* 119:609–614.

Chiocca, E. A., Davies, P. J., and Stein, J. P. (1989): Regulation of tissue transglutaminase gene expression as a molecular model for retinoid effects on proliferation and differentiation. *J. Cell Biochem.* 39:293–304.

Chivot, M., and Midoun, H. (1990): Isotretinoin and acne—a study of relapses. *Dermatologica* 180:240–243.

Connor, M. J., and Smit, M. H. (1987): Terminal-group oxidation of retinol by mouse epidermis. Inhibition *in vitro* and *in vivo*. *Biochem. J.* 244:489–492.

Cooper, T. W., Tabas, M., and Bauer, E. A. (1985): Retinoic acid in recessive dystrophic epidermolysis bullosa- *in vitro* effects on collagenase and preliminary therapeutic trials. In: *Retinoids: New trends in research and therapy*, edited by J.-H. Saurat. pp. 219–224. Karger, Basel, New York.

Costa, A., Malone, W., Perloff, M., Buranelli, F., Campa, T., Dossena, G., Magni, A., Pizzichetta, M., Andreoli, C., Del Vecchio, M., Formelli, F., and Barbieri, A. (1989): Tolerability of the synthetic retinoid Fenretinide (HPR). *Eur. J. Cancer Clin. Oncol.* 25:805–808.

Cunliffe, W. J., Layton, A., Knaggs, H. E., Stubbings, J., and Taylor, J. P. (1991): Isotretinoin and acne: a long-term study. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 274–280. Karger, Basel, New York.

Cunliffe, W. J., and Norris, J. F. B. (1987): Isotretinoin—an explanation for its long-term benefit. *Dermatologica* 175[Suppl 1]:133–137.

Dai, W. S., LaBraico, J. M., and Stern, R. S. (1992): Epidemiology of isotretinoin exposure during pregnancy. *J. Am. Acad. Dermatol.* 26:599–606.

DeLuca, L. M. (1978): Vitamin A. In: *Handbook of lipid research*, Vol 2, edited by L. M. DeLuca. p. 1. Plenum Publishing, New York.

DeLuca, L. M. (1977): Epithelial membranes and vitamin A. In: *Mammalian cell membranes*, edited by G. A. Jamieson, D. M. Robinson. p. 231. Butterworth, Boston.

Dennert, G. (1984): Retinoids and the immune system: Immunostimulation by vitamin A. In: *The retinoids*, Vol 2, edited by M. B. Sporn, A. B. Roberts, and D. S. Goodman. p. 373. Academic Press, New York.

Dicken, C. H., and Connolly, S. M. (1980): Eruptive xanthomas associated with isotretinoin (13-*cis*-retinoic acid). *Arch. Dermatol.* 116:951–952.

DiGiovanna, J. J., Helfgott, R. K., Gerber, L. H., and Peck, G. L. (1986): Extraspinal tendon and ligament calcification associated with long-term therapy with etretinate. *N. Engl. J. Med.* 315:1177–1182.

DiGiovanna, J. J., Zech, L. A., Ruddel, M. E., Gantt, G., and Peck, G. L. (1989): Etretinate. Persistent serum levels after long-term therapy. *Arch. Dermatol.* 125:246–251.

Dingle, J. T. (1968): Vacuoles, vesicles and lysosomes. *Br. Med. Bull.* 24:141–145.

Doran, T. I., Lucas, D. A., Levin, A. A., Pacia, E., Sturzenbecker, L., Allenby, G., Grippo, J. F., and Shapiro, S. S. (1991): Biochemical and retinoid receptor activities in human sebaceous cells. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 243–253. Karger, Basel, New York.

Doran, T. I., and Shapiro, S. S. (1990): Retinoid effects on sebocyte proliferation. *Methods Enzymol.* 190:334–338.

Dubertret, L., Lebreton, C., and Touraine, R. (1982): Inhibition of neutrophil migration by etretinate and its main metabolite. *Br. J. Dermatol.* 107:681–685.

Dupuy, P., Bagot, M., Heslan, M., and Dubertret, L. (1989): Synthetic retinoids inhibit the antigen presenting properties of epidermal cells in vitro. *J. Invest. Dermatol.* 93:455–459.

Ehmann, C. W., and Voorhees, J. J. (1982): International studies of the efficacy of etretinate in the treatment of psoriasis. *J. Am. Acad. Dermatol.* 6:692–696.

Eichner, R., Kahn, M., Capetola, R. J., Gendimenico, G. J., and Mezick, J. A. (1992): Effects of topical retinoids on cytoskeletal proteins: implications for retinoid effects on epidermal differentiation. *J. Invest. Dermatol.* 98:154–161.

Elias, P. M., and Friend, D. S. (1976): Vitamin-A-induced mucous metaplasia. An *in vitro* system for modulating tight- and gap-junction differentiation. *J. Cell Biol.* 68:173–188.

Elias, P. M., Fritsch, P. O., Lampe, M., Williams, M. L., Brown, B. E., Nemanic, M., and Grayson, S. (1981a): Retinoid effects on epidermal structure, differentiation, and permeability. *Lab. Invest.* 44:531–540.

Elias, P. M., Grayson, S., Gross, E. G., Peck, G. L., and McNutt, N. S. (1981b): Influence of topical and systemic retinoids on basal cell carcinoma cell membranes. *Cancer* 48:932–938.

Elias, P. M., Chung, J. C., Orozco-Topete, R., and Nemanic, M. K. (1983): Membrane glycoconjugate visualization and biosynthesis in normal and retinoid-treated epidermis. *J. Invest. Dermatol.* 81:81s–85s.

Elias, P. M., and Williams, M. L. (1981): Retinoids, cancer, and the skin. *Arch. Dermatol.* 117:160–168.

Ellis, C. N., Gold, R. C., Grekin, R. C., Anderson, T. F., Swanson, N. A., and Voorhees, J. J. (1982): Etretinate therapy stimulates deposition of mucus-like material in epidermis of patients with psoriasis. *J. Am. Acad. Dermatol.* 6:699–704.

Ellis, C. N., Madison, K. C., Pennes, D. R., Martel, W., and Voorhees, J. J. (1984): Isotretinoin therapy is associated with early skeletal radiographic changes. *J. Am. Acad. Dermatol.* 10:1024–1029.

Ellis, C. N., Pennes, D. R., Hermann, R. C., Blauvelt, A., Martel, W., and Voorhees, J. J. (1988): Long-term radiographic follow-up after isotretinoin therapy. *J. Am. Acad. Dermatol.* 18:1252–1261.

Ensink, B. W., and van Voorst Vader, P. C. (1983): Ophthalmological side-effects of 13-*cis*-retinoid therapy. *Br. J. Dermatol.* 108:627.

Eriksen, L., and Cormane, R. H. (1975): Oral retinoic acid as therapy for congenital ichthyosiform erythroderma. *Br. J. Dermatol.* 92:343–345.

Exner, J. H., Dahod, S., and Pochi, P. E. (1983): Pyogenic granuloma-like acne lesions during isotretinoin therapy. *Arch. Dermatol.* 119:808–811.

Fallon, M. B., and Boyer, J. L. (1990): Hepatic toxicity of vitamin A and synthetic retinoids. *J. Gastroenterol. Hepatol.* 5:334–342.

Farb, R. M., Lazarus, G. S., Chiaramonti, A., Goldsmith, L. A., Gilgor, R. S., and Balakrishnan, C. V. (1980): The effect of 13-*cis*-retinoic acid on epidermal lysosomal hydrolase activity in Darier's disease and pityriasis rubra pilaris. *J. Invest. Dermatol.* 75:133–135.

Farrell, L. N., Strauss, J. S., and Stranieri, A. M. (1980): The treatment of severe cystic acne with 13-*cis*-retinoic acid. Evaluation of sebum production and the clinical response in a multiple-dose trial. *J. Am. Acad. Dermatol.* 3:602–611.

Fell, H. B., and Mellanby, E. (1953): Metaplasia produced in cultures of chick ectoderm by high vitamin A. *J. Physiol.* 119:470–488.

Floyd, E. E., and Jetten, A. M. (1989): Regulation of type I (epidermal) transglutaminase mRNA levels during squamous differentiation: downregulation by retinoids. *Mol. Cell Biol.* 9:4846–4851.

Frame, B., Jackson, C. E., Reynolds, W. A., and Umphrey, J. E. (1974): Hypercalcemia and skeletal effects in chronic hypervitaminosis A. *Ann. Intern. Med.* 80:44–48.

Fritsch, P. (1981): Oral retinoids in dermatology. *Int. J. Dermatol.* 20:314–329.

Fritsch, P. O., Hönigsmann, H., Jaschke, E., and Wolff, K. (1978): Augmentation of oral methoxsalen-photochemotherapy with an oral retinoic acid derivative. *J. Invest. Dermatol.* 70:178–182.

Fritsch, P. O., Pohlin, G., Längle, U., and Elias, P. M. (1981): Response of epidermal cell proliferation to orally administered aromatic retinoid. *J. Invest. Dermatol.* 77:287–291.

Frolik, C. A. (1981): *In vitro* and *in vivo* metabolism of all-*trans*- and 13-*cis*-retinoic acid in the hamster. In: *Modulation of cellular interaction by vitamin A and derivatives (retinoids)*, edited by L. M. DeLuca and S. S. Shapiro. pp. 37. The New York Academy of Sciences, New York.

Fuchs, E., and Green, H. (1981): Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. *Cell* 25:617–625.

Fumarulo, R., Conese, M., Riccardi, S., Giordano, D., Montemurro, P., Colucci, M., and Semeraro, N. (1991): Retinoids inhibit the respiratory burst and degranulation of stimulated human polymorphonuclear leukocytes. *Agents Actions* 34:339–344.

Geesin, J. C., Gordon, J. S., and Berg, R. A. (1990): Retinoids affect collagen synthesis through inhibition of ascorbate-induced lipid peroxidation in cultured human dermal fibroblasts. *Arch. Biochem. Biophys.* 278:350–355.

Gerber, L. H., Helfgott, R. K., Gross, E. G., Hicks, J. E., Ellenberg, S. S., and Peck, G. L. (1984): Vertebral abnormalities associated with synthetic retinoid use. *J. Am. Acad. Dermatol.* 10:817–823.

Gilfix, B. M., and Eckert, R. L. (1985): Coordinate control by vitamin A of keratin gene expression in human keratinocytes. *J. Biol. Chem.* 260:14026–14029.

Godfrey, K. M., and James, M. P. (1990): Treatment of severe acne with isotretinoin in patients with inflammatory bowel disease. *Br. J. Dermatol.* 123:653–655.

Goerz, G., and Orfanos, C. E. (1978): Systemic treatment of psoriasis with a new aromatic retinoid. Preliminary evaluation of a multicenter controlled study in the Federal Republic of Germany. *Dermatologica* 157[Suppl 1]:38–44.

Gold, J. A., Shupack, J. L., and Nemec, M. A. (1989): Ocular side effects of the retinoids. *Int. J. Dermatol.* 28:218–225.

Goldberg, L. H., Hsu, S. H., and Alcalay, J. (1989): Effectiveness of isotretinoin in preventing the appearance of basal cell carcinomas in basal cell nevus syndrome. *J. Am. Acad. Dermatol.* 21:144–145.

Goldsmith, L. A., Weinrich, A. E., and Shupack, J. (1982): Pityriasis rubra pilaris response to 13-*cis*-retinoic acid (isotretinoin). *J. Am. Acad. Dermatol.* 6:710–715.

Goldstein, J. A., Socha-Szott, A., Thomsen, R. J., Pochi, P. E., Shalita, A. R., and Strauss, J. S. (1982): Comparative effect of isotretinoin and etretinate on acne and sebaceous gland secretion. *J. Am. Acad. Dermatol.* 6:760–765.

Gollnick, H. (1991): Acitretin in psoriasis: an update. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 204–213. Karger, Basel, New York.

Gomez, E. C. (1981): Differential effect of 13-*cis*-retinoic acid and an aromatic retinoid (Ro 10-9359) on the sebaceous glands of the hamster flank organ. *J. Invest. Dermatol.* 76:68–69.

Grekin, R. C., Ellis, C. N., Goldstein, N. G., Swanson, N. A., Anderson, T. F., Duell, E. A., and Voorhees, J. J. (1983): Decreased urinary polyamines in patients with psoriasis treated with etretinate. *J. Invest. Dermatol.* 80:181–184.

Griffiths, C. E. M., Rosenthal, D. S., Reddy, A. P., Elder, J. T., Astrom, A., Leach, K., Wang, T. S., Finkel, L. J., Yuspa, S. H., Voorhees, J. J., and Fisher, G. J. (1992): Short-term retinoic acid treatment increases *in vivo*, but decreases *in vitro*, epidermal transglutaminase-K enzyme activity and immunoreactivity. *J. Invest. Dermatol.* 99:283–288.

Grupper, C., and Berretti, B. (1981): Treatment of psoriasis by oral PUVA-therapy combined with aromatic retinoid (Re-PUVA). In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. pp. 341. Springer-Verlag, New York.

Guo, H., Acevedo, P., Parsa, F. D., and Bertram, J. S. (1992): Gap-junctional protein connexin 43 is expressed in dermis and epidermis of human skin: differential modulation by retinoids. *J. Invest. Dermatol.* 99:460–467.

Haftek, M., Faure, M., Schmitt, D., and Thivolet, J. (1983): Langerhans cells in skin from patients with psoriasis: quantitative and qualitative study of T6 and HLA-DR antigen-expressing cells and changes with aromatic retinoid administration. *J. Invest. Dermatol.* 81:10–14.

Halliday, G. M., Ho, K. K., and Barnetson, R. S. (1992): Regulation of the skin immune system by retinoids during carcinogenesis. *J. Invest. Dermatol.* 99:83S–86S.

Harms, M., Masouyë, I., and Radeff, B. (1986): The relapses of cystic acne after isotretinoin treatment are age-related: a long-term follow-up study. *Dermatologica* 172:148–153.

Hassell, J. R., Pennypacker, J. P., Kleinman, H. K., Pratt, R. M., and Yamada, K. M. (1979): Enhanced cellular fibronectin accumulation in chondrocytes treated with vitamin A. *Cell* 17:821–826.

Haydey, R. P., Reed, M. L., Dzubow, L. M., and Shupack, J. L. (1980): Treatment of keratoacanthomas with oral 13-*cis*-retinoic acid. *N. Engl. J. Med.* 303:560–562.

Hodak, E., Ginzburg, A., David, M., and Sandbank, M. (1987): Etretinate treatment of the nevoid basal cell carcinoma syndrome. Therapeutic and chemopreventive effect. *Int. J. Dermatol.* 26:606–609.

Hohl, D., Lichti, U., Breitkreutz, D., Steinert, P. M., and Roop, D. R. (1991): Transcription of the human loricrin gene *in vitro* is induced by calcium and cell density and suppressed by retinoic acid. *J. Invest. Dermatol.* 96:414–418.

Holland, D. B., Gowland, G., and Cunliffe, W. J. (1984): Inflammatory

responses in acne patients treated with 13-*cis*-retinoic acid (isotretinoin). *Br. J. Dermatol.* 110:343–345.

Hong, W. K., Endicott, J., Itri, L. M., Doos, W., Batsakis, J. G., Bell, R., Fofonoff, S., Byers, R., Atkinson, E. N., and Vaughan, C. (1986): 13-*cis*-retinoic acid in the treatment of oral leukoplakia. *N. Engl. J. Med.* 315:1501–1505.

Hong, W. K., Lippman, S. M., Itri, L. M., Karp, D. D., Lee, J. S., Byers, R. M., Schantz, S. P., Kramer, A. M., Lotan, R., and Peters, L. J. (1990): Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* 323:795–801.

Hönigsmann, H., and Wolff, K. (1983): Isotretinoin-PUVA for psoriasis. *Lancet* 1:236.

Hönigsmann, H., and Wolff, K. (1989): Results of therapy for psoriasis using retinoid and photochemotherapy (RePUVA). *Pharmacol. Ther.* 40:67–73.

Huang, M. E., Ye, Y. C., Chen, S. R., Chai, J. R., Lu, J. X., Zhoa, L., Gu, L. J., and Wang, Z. Y. (1988): Use of all-*trans*-retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 72:567–572.

Jetten, A. M., Jetten, M. E., Shapiro, S. S., and Poon, J. P. (1979): Characterization of the action of retinoids on mouse fibroblast cell lines. *Exp. Cell Res.* 119:289–299.

Jetten, A. M. (1984): Modulation of cell growth by retinoids and their possible mechanisms of action. *Fed. Proc.* 43:134–139.

Jones, D. H., King, K., Miller, A. J., and Cunliffe, W. J. (1983): A dose-response study of 13-*cis*-retinoic acid in acne vulgaris. *Br. J. Dermatol.* 108:333–343.

Kaiser-Kupfer, M. I., Peck, G. L., Caruso, R. C., Jaffe, M. J., DiGiovanna, J. J., and Gross, E. G. (1986): Abnormal retinal function associated with fenretinide, a synthetic retinoid. *Arch. Ophthalmol.* 104:69–70.

Kaplan, R. P., Russell, D. H., and Lowe, N. J. (1983): Etretinate therapy for psoriasis: clinical responses, remission times, epidermal DNA and polyamine responses. *J. Am. Acad. Dermatol.* 8:95–102.

Katz, R. A., Jorgensen, H., and Nigra, T. P. (1983): Flare of cystic acne from oral isotretinoin. *J. Am. Acad. Dermatol.* 8:132–133.

Kessler, J. F., Meyskens, F. L., Jr., Levine, N., Lynch, P. J., and Jones, S. E. (1983): Treatment of cutaneous T-cell lymphoma (mycosis fungoides) with 13-*cis*-retinoic acid. *Lancet* 1:1345–1347.

Kilcoyne, R. F., Cope, R., Cunningham, W., Nardella, F. A., Denman, S., Franz, T. J., and Hanifin, J. (1986): Minimal spinal hyperostosis with low-dose isotretinoin therapy. *Invest. Radiol.* 21:41–44.

Kilcoyne, R. F. (1988): Effects of retinoids in bone. *J. Am. Acad. Dermatol.* 19:212–216.

King, K., Jones, D. H., Daltrey, D. C., and Cunliffe, W. J. (1982): A double-blind study of the effects of 13-*cis*-retinoic acid on acne, sebum excretion rate and microbial population. *Br. J. Dermatol.* 107:583–590.

Kingston, T. P., Lowe, N. J., Winston, J., and Heckenlively, J. (1986): Visual and cutaneous toxicity which occurs during *N*-(4-hydroxyphenyl) retinamide therapy for psoriasis. *Clin. Exp. Dermatol.* 11:624–627.

Kitajima, Y., and Mori, S. (1983): Effects of retinoid (Ro 10-9359) on the plasma membrane of keratinocytes in patients with psoriasis: a freeze-fracture analysis. *J. Invest. Dermatol.* 80:174–180.

Kligman, A. M., Fulton, J. E., Jr., and Plewig, G. (1969): Topical vitamin A acid in acne vulgaris. *Arch. Dermatol.* 99:469–476.

Klinkhoff, A. V., Gertner, E., Chalmers, A., Gladman, D. D., Stewart, W. D., Schachter, G. D., and Schachter, R. K. (1989): Pilot study of etretinate in psoriatic arthritis. *J. Rheumatol.* 16:789–791.

Koch, H. F. (1981): Effect of retinoids on precancerous lesions of oral mucosa. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 307. Springer-Verlag, New York.

Kraemer, K. H., Lee, M. M., and Scotto, J. (1987): Xeroderma pigmentosum: cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch. Dermatol.* 123:241–250.

Kraemer, K. H., DiGiovanna, J. J., Moshell, A. N., Tarone, R. E., and Peck, G. L. (1988): Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. *N. Engl. J. Med.* 318:1633–1637.

Kraemer, K. H., DiGiovanna, J. J., Moshell, A. N., Tarone, R. E., and Peck, G. L. (1990): Oral isotretinoin prevention of skin cancer in xeroderma pigmentosum: individual variation in dose response. *J. Invest. Dermatol.* 94:544.

Lambert, W. E., Meyer, E., De Leenheer, A. P., De Bersaques, J., and Kint, A. H. (1992): Pharmacokinetics and drug interactions of etretinate and acitretin. *J. Am. Acad. Dermatol.* 27:S19–S22.

Lammer, E. J., Chen, D. T., Hoar, R. M., Agnish, N. D., Benke, P. J., Braun, J. T., Curry, C. J., Fernhoff, P. M., Grix, A. W., Jr., and Lott, I. T. (1985): Retinoic acid embryopathy. *N. Engl. J. Med.* 313:837–841.

Lammer, E. J. (1988): Embryopathy in infant conceived one year after termination of maternal etretinate. *Lancet* 2:1080–1081.

Landthaler, M., Kummermehr, J., Wagner, A., Nikolowski, J., and Plewig, G. (1981): Effects of 13-*cis*-retinoic acid on sebaceous glands in humans. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 259. Springer-Verlag, New York.

Lauharanta, J., Juvakoski, T., Kanerva, L., and Lassus, A. (1981a): Aromatic retinoid (Ro 10-9359), Re-PUVA, and PUVA in the treatment of psoriasis. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. pp. 201–203. Springer-Verlag, New York.

Lauharanta, J., Kousa, M., Kapyaho, K., Linnamaa, K., and Mustakallio, K. (1981b): Reduction of increased polyamine levels in psoriatic lesions by retinoid and PUVA treatments. *Br. J. Dermatol.* 105:267–272.

Laurberg, G., Geiger, J. M., Hjorth, N., Holm, P., Hou-Jensen, K., Jacobsen, K. U., Nielsen, A. O., Pichard, J., Serup, J., and Sparre-Jorgensen, A. (1991): Treatment of lichen planus with acitretin. A double-blind, placebo-controlled study in 65 patients. *J. Am. Acad. Dermatol.* 24:434–437.

Law, W. C., and Rando, R. R. (1989): The molecular basis of retinoic acid induced night blindness. *Biochem. Biophys. Res. Commun.* 161:825–829.

Lawlor, F., and Peiris, S. (1985): Harlequin fetus successfully treated with etretinate. *Br. J. Dermatol.* 112:585–590.

Lawson, J. P., and McGuire, J. (1987): The spectrum of skeletal changes associated with long-term administration of 13-*cis*-retinoic acid. *Skeletal. Radiol.* 16:91–97.

Lazar, A. P., and Lazar, P. (1986): Cowden's disease (multiple hamartoma and neoplasia syndrome) treated with isotretinoin. *J. Am. Acad. Dermatol.* 14:142–144.

Lazarus, G. S., Hatcher, V. B., and Levine, N. (1975): Lysosomes and the skin. *J. Invest. Dermatol.* 65:259–271.

Levine, N., and Meyskens, F. L., Jr. (1980): Topical vitamin-A-acid therapy for cutaneous metastatic melanoma. *Lancet* 2:224–226.

Leyden, J. J., and McGinley, K. J. (1982): Effect of 13-cis-retinoic acid on sebum production and *Propionibacterium acnes* in severe nodulocystic acne. *Arch. Dermatol. Res.* 272:331–337.

Liauw, L., Lim, D. J., Bakaletz, L. O., and van Blitterswijk, C. (1991): Effect of vitamin A on the growth and differentiation of rat external auditory canal epithelium in organ culture. *Am. J. Otolaryngol.* 12:67–75.

Lippman, S. M., and Meyskens, F. L., Jr. (1987): Treatment of advanced squamous cell carcinoma of the skin with isotretinoin. *Ann. Intern. Med.* 107:499–502.

Lippman, S. M., and Meyskens, F. L., Jr. (1989): Results of the use of vitamin A and retinoids in cutaneous malignancies. *Pharmacol. Ther.* 40:107–122.

Lippman, S. M., Parkinson, D. R., Itri, L. M., Weber, R. S., Schantz, S. P., Ota, D. M., Schusterman, M. A., Krakoff, I. H., Gutterman, J. U., and Hong, W. K. (1992): 13-*cis*-Retinoic acid and interferon alpha-2a: effective combination therapy for advanced squamous cell carcinoma of the skin. *J. Natl. Cancer Inst.* 84:235–241.

Lookingbill, D. P., Demers, L. M., Tigelaar, R. E., and Shalita, A. R. (1988): Effect of isotretinoin on serum levels of precursor and peripherally derived androgens in patients with acne. *Arch. Dermatol.* 124:540–543.

Lotan, R., Kramer, R. H., Neumann, G., Lotan, D., and Nicolson, G. L. (1980): Retinoic acid-induced modifications in the growth and cell-surface components of a human carcinoma (HeLa) cell line. *Exp. Cell Res.* 130:401–414.

Lowe, N. J., Kaplan, R., and Breeding, J. (1982): Etretinate treatment for psoriasis inhibits epidermal ornithine decarboxylase. *J. Am. Acad. Dermatol.* 6:697–698.

Lowe, N. J. (1991): When systemic retinoids fail to work in psoriasis.

In: *Retinoids: 10 years on*, edited by J.-H. Saurat, pp. 341–349. Karger, Basel, New York.
Löwhagen, G. B., Michaëlsson, G., Mobacken, H., Pettersson, U., and Vahlquist, A. (1982): Effects of etretinate (Ro 10-9359) on Darier's disease. *Dermatologica* 165:123–130.
Lutzner, M. A., and Blanchet-Bardon, C. (1980): Oral retinoid treatment of human papillomavirus type 5-induced epidermodysplasia verruciformis. *N. Engl. J. Med.* 302:1091.
Mahrle, G., Meyer-Hamme, S., and Ippen, H. (1982): Oral treatment of keratinizing disorders of skin and mucous membranes with etretinate. Comparative study of 113 patients. *Arch. Dermatol.* 118:97–100.
Marsden, J. R. (1989): Lipid metabolism and retinoid therapy. *Pharmacol. Ther.* 40:55–65.
Matsuoka, L. Y., Wortsman, J., Lifrak, E. T., Parker, L. N., and Mehta, R. G. (1989): Effect of isotretinoin in acne is not mediated by adrenal androgens. *J. Am. Acad. Dermatol.* 20:128–129.
McGuire, J., Fedarko, N., Johanssen, E., La Vigne, J., Lyons, G., Milstone, L., and Osber, M. (1982): The influence of retinoids on cultivated human keratinocytes. *J. Am. Acad. Dermatol.* 6:630–639.
Meigel, W., Gollnick, H., Wokalek, H., and Plewig, G. (1983): Oral treatment of acne conglobata using 13-*cis*-retinoic acid. Results of the German multicentric study following 24 weeks of treatment. *Hautarzt.* 34:387–397.
Milstone, L. M., McGuire, J., and Ablow, R. C. (1982): Premature epiphyseal closure in a child receiving oral 13-*cis*-retinoic acid. *J. Am. Acad. Dermatol.* 7:663–666.
Moriarty, M., Dunn, J., Darragh, A., Lambe, R., and Brick, I. (1982): Etretinate in treatment of actinic keratosis. A double-blind crossover study. *Lancet* 1:364–365.
Nakanishi, K., Nara, K., Hagiwara, H., Aoyama, Y., Ueno, H., and Hirose, S. (1991): Cloning and sequence analysis of cDNA clones for bovine aortic-endothelial-cell transglutaminase. *Eur. J. Biochem.* 202:15–21.
Nemanic, M. K., Fritsch, P. O., and Elias, P. M. (1982): Perturbations of membrane glycosylation in retinoid-treated epidermis. *J. Am. Acad. Dermatol.* 6:801–808.
Nervi, C., Vollberg, T. M., George, M. D., Zelent, A., Chambon, P., and Jetten, A. M. (1991): Expression of nuclear retinoic acid receptors in normal tracheobronchial cells and in lung carcinoma cells. *Exp. Cell Res.* 195:163–170.
Norris, D. A., Osborn, R., Robinson, W., and Tonnesen, M. G. (1987): Isotretinoin produces significant inhibition of monocyte and neutrophil chemotaxis *in vivo* in patients with cystic acne. *J. Invest. Dermatol.* 89:38–43.
O'Brien, T. G., Simsiman, R. C., and Boutwell, R. K. (1975): Induction of the polyamine-biosynthetic enzymes in mouse epidermis and their specificity for tumor promotion. *Cancer Res.* 35:2426–2433.
O'Brien, T. G. (1976): The induction of ornithine decarboxylase as an early, possibly obligatory, event in mouse skin carcinogenesis. *Cancer Res.* 36:2644–2653.
Oikarinen, A. (1989): Comparison of the effects of retinoids and glucocorticosteroid on protein and type IV collagen synthesis in HT-1080 (human basement membrane forming fibrosarcoma) cells. *Dermatologica* 179:14–17.
Orfanos, C. E., Schmidt, H. W., Mahrle, G., and Runne, U. (1972): Effect of vitamin A acid (VAA) on psoriasis. Topical combined therapy using corticoids. Two new VAA preparations for oral administration. *Arch. Dermatol. Forsch.* 244:424–426.
Orfanos, C. E., Steigleder, G. K., Pullmann, H., and Bloch, P. H. (1979): Oral retinoid and UVB radiation: a new, alternative treatment for psoriasis on an out-patient basis. *Acta Derm. Venereol. (Stockh)* 59:241–244.
Orfanos, C. E. (1980): Oral retinoids—present status. *Br. J. Dermatol.* 103:473–481.
Orfanos, C. E., Gollnick, H., and Tsambaos, D. (1981): New aspects and developments in antipsoriasis retinoid therapy. *Hautarzt.* 32:275–280.
Orfanos, C. E., Ehlert, R., and Gollnick, H. (1987): The retinoids. A review of their clinical pharmacology and therapeutic use. *Drugs* 34:459–503.
Orfanos, C. E., and Runne, U. (1976): Systemic use of a new retinoid with and without local dithranol treatment in generalized psoriasis. *Br. J. Dermatol.* 95:101–103.
Ott, F., and Bollag, W. (1975): Treatment of psoriasis with an orally administered effective new vitamin A acid derivative. Preliminary report. *Schweiz. Med. Wochenschr.* 105:439–441.
Patt, L. M., Itaya, K., and Hakomori, S. I. (1978): Retinol induces density-dependent growth inhibition and changes in glycolipids and LETS. *Nature* 273:379–381.
Peck, G. L., Elias, P. M., and Wetzel, B. (1977): Effects of retinoic acid on embryonic chick skin. *J. Invest. Dermatol.* 69:463–476.
Peck, G. L., Yoder, F. W., Olsen, T. G., Pandya, M. D., and Butkus, D. (1978): Treatment of Darier's disease, lamellar ichthyosis, pityriasis rubra pilaris, cystic acne, and basal cell carcinoma with oral 13-*cis*-retinoic acid. *Dermatologica* 157[Suppl 1]:11–12.
Peck, G. L., Olsen, T. G., Yoder, F. W., Strauss, J. S., Downing, D. T., Pandya, M., Butkus, D., and Arnaud-Battandier, J. (1979): Prolonged remissions of cystic and conglobate acne with 13-*cis*-retinoic acid. *N. Engl. J. Med.* 300:329–333.
Peck, G. L., Gross, E. G., and Butkus, D. (1981): Comparative analysis of two retinoids in the treatment of disorders of keratinization. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli, pp. 279–286. Springer-Verlag, New York.
Peck, G. L., Gross, E. G., Butkus, D., and DiGiovanna, J. J. (1982a): Chemoprevention of basal cell carcinoma with isotretinoin. *J. Am. Acad. Dermatol.* 6:815–823.
Peck, G. L., Olsen, T. G., Butkus, D., Pandya, M., Arnaud-Battandier, J., Gross, E. G., Windhorst, D. B., and Cheripko, J. (1982b): Isotretinoin versus placebo in the treatment of cystic acne. A randomized double-blind study. *J. Am. Acad. Dermatol.* 6:735–745.
Peck, G. L. (1987): Long-term retinoid therapy is needed for maintenance of cancer chemopreventive effect. *Dermatologica* 175[Suppl 1]:138–144.
Peck, G. L., DiGiovanna, J. J., Sarnoff, D. S., Gross, E. G., Butkus, D., Olsen, T. G., and Yoder, F. W. (1988): Treatment and prevention of basal cell carcinoma with oral isotretinoin. *J. Am. Acad. Dermatol.* 19:176–185.
Peck, G. L., and Yoder, F. W. (1976): Treatment of lamellar ichthyosis and other keratinising dermatoses with an oral synthetic retinoid. *Lancet* 2:1172–1174.
Peck, S. M., Chargin, L., and Sobotka, H. (1941): Keratosis follicularis (Darier's disease)—a vitamin A-deficiency disease. *Arch. Dermatol. Syphilol.* 43:223.
Peng, Y. M., Dalton, W. S., Alberts, D. S., Xu, M. J., Lim, H., and Meyskens, F. L., Jr. (1989): Pharmacokinetics of N-4-hydroxyphenyl-retinamide and the effect of its oral administration on plasma retinol concentrations in cancer patients. *Int. J. Cancer* 43:22–26.
Pennes, D. R., Martel, W., and Ellis, C. N. (1985): Retinoid-induced ossification of the posterior longitudinal ligament. *Skelet. Radiol.* 14:191–193.
Perkins, W., Crocket, K. V., Hodgins, M. B., MacKie, R. M., and Lackie, J. M. (1991): The effect of treatment with 13-*cis*-retinoic acid on the metabolic burst of peripheral blood neutrophils from patients with acne. *Br. J. Dermatol.* 124:429–432.
Pittsley, R. A., and Yoder, F. W. (1983): Retinoid hyperostosis. Skeletal toxicity associated with long-term administration of 13-*cis*-retinoic acid for refractory ichthyosis. *N. Engl. J. Med.* 308:1012–1014.
Plewig, G., Nikolowski, J., and Wolff, H. H. (1982): Action of isotretinoin in acne rosacea and gram-negative folliculitis. *J. Am. Acad. Dermatol.* 6:766–785.
Plewig, G., Wagner, A., Nikolowski, J., and Landthaler, M. (1981): Effects of two retinoids in animal experiments and after clinical application in acne patients: 13-cis-retinoic acid Ro 4-3780 and aromatic retinoid Ro 10-9359. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli, pp. 219–235. Springer-Verlag, New York.
Poddar, S., Hong, W. K., Thacher, S. M., and Lotan, R. (1991): Retinoic acid suppression of squamous differentiation in human head-and-neck squamous carcinoma cells. *Int. J. Cancer* 48:239–247.
Porter, A. D. (1951): Vitamin A in some congenital anomalies of the skin. *Br. J. Dermatol.* 63:123.
Prabhala, R. H., Garewal, H. S., Hicks, M. J., Sampliner, R. E., and Watson, R. R. (1991): The effects of 13-*cis*-retinoic acid and beta-carotene on cellular immunity in humans. *Cancer* 67:1556–1560.
Prutkin, L. (1975): Mucous metaplasia and gap junctions in the vita-

min A acid-treated skin tumor, keratoacanthoma. *Cancer Res.* 35:364–369.

Rademaker, M., Wallace, M., Cunliffe, W., and Simpson, N. B. (1991): Isotretinoin treatment alters steroid metabolism in women with acne. *Br. J. Dermatol.* 124:361–364.

Rice, R. H., Cline, P. R., and Coe, E. L. (1983): Mutually antagonistic effects of hydrocortisone and retinyl acetate on envelope competence in cultured malignant human keratinocytes. *J. Invest. Dermatol.* 81:176s–178s.

Roberts, L. J. (1989): Long-term survival of a harlequin fetus. *J. Am. Acad. Dermatol.* 21:335–339.

Rodland, O., Aksnes, L., Nilsen, A., and Morken, T. (1992): Serum levels of vitamin D metabolites in isotretinoin-treated acne patients. *Acta Derm. Venereol. (Stockh)* 72:217–219.

Roels, O. A., Anderson, O. R., Lui, N. S., Shah, D. O., and Trout, M. E. (1969): Vitamin A and membranes. *Am. J. Clin. Nutr.* 22:1020–1032.

Roenigk, H. H., Jr. (1989): Liver toxicity of retinoid therapy. *Pharmacol. Ther.* 40:145–155.

Rosenthal, D. S., Griffiths, C. E. M., Yuspa, S. H., Roop, D. R., and Voorhees, J. J. (1992): Acute or chronic topical retinoic acid treatment of human skin *in vivo* alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin, and keratins 6 and 13 but not keratins 1, 10, and 14. *J. Invest. Dermatol.* 98:343–350.

Rubinow, D. R., Peck, G. L., Squillace, K. M., and Gantt, G. G. (1987): Reduced anxiety and depression in cystic acne patients after successful treatment with oral isotretinoin. *J. Am. Acad. Dermatol.* 17:25–32.

Rustin, G. J. S., Dische, S., de Garis, S. T., and Nelstrop, A. (1988): Treatment of advanced malignant melanoma with interferon alpha and etretinate. *Eur. J. Cancer Clin. Oncol.* 24:783–784.

Ruzicka, T., Meurer, M., and Bieber, T. (1988): Efficiency of acitretin in the treatment of cutaneous lupus erythematosus. *Arch. Dermatol.* 124:897–902.

Safran, A. B., Halioua, B., Roth, A., and Saurat, J.-H. (1991): Ocular side effects of oral treatment with retinoids. A review of clinical findings and molecular mechanisms and a prospective study of the influence of acitretin on retinal function. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 315–326. Karger, Basel, New York.

Sanchez-Conejo-Mir, J., and Camacho, F. (1989): Nevoid basal cell carcinoma syndrome: combined etretinate and surgical treatment. *J. Dermatol. Surg. Oncol.* 15:868–871.

Schill, W.-B., Wagner, A., Nikolowski, J., and Plewig, G. (1981): Aromatic retinoid and 13-*cis*-retinoic acid: spermatological investigations. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 389. Springer-Verlag, New York.

Schmidt, R., Cathelineau, C., Cavey, M. T., Dionisius, V., Michel, S., Shroot, B., and Reichert, U. (1989): Sodium butyrate selectively antagonizes the inhibitory effect of retinoids on cornified envelope formation in cultured human keratinocytes. *J. Cell Physiol.* 140:281–287.

Schnitzler, L., and Verret, J. L. (1981): Retinoid and skin cancer prevention. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 385. Springer-Verlag, New York.

Seawright, A. A., and English, P. B. (1967): Hypervitaminosis A and deforming cervical spondylosis of the cat. *J. Comp. Pathol.* 77:29–39.

Shalita, A. R., Cunningham, W. J., Leyden, J. J., Pochi, P. E., and Strauss, J. S. (1983): Isotretinoin treatment of acne and related disorders: an update. *J. Am. Acad. Dermatol.* 9:629–638.

Shapiro, S. S., and Poon, J. P. (1979): Retinoic acid-induced alterations of growth and morphology in an established epithelial line. *Exp. Cell Res.* 119:349–357.

Sherman, M. I., Strickland, S., and Reich, E. (1976): Differentiation of early mouse embryonic and teratocarcinoma cells *in vitro*: plasminogen activator production. *Cancer Res.* 36:4208–4216.

Shornick, J. K., Formica, N., and Parke, A. L. (1991): Isotretinoin for refractory lupus erythematosus. *J. Am. Acad. Dermatol.* 24:49–52.

Siegenthaler, G., and Saurat, J.-H. (1991): Natural retinoids: metabolism and transport in human epidermal cells. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 56–68. Karger, Basel, New York.

Slawsky, L. D., and Libow, L. F. (1990): Successful treatment of acrodermatitis continua of Hallopeau with etretinate. *J. Am. Acad. Dermatol.* 23:1176–1178.

Sofen, H. L., Moy, R. L., and Lowe, N. J. (1984): Treatment of generalised pustular psoriasis with isotretinoin. *Lancet* 1:40.

Souteyrand, P., Thivolet, J., and Fulton, R. (1981): Treatment of parapsoriasis en plaques and mycosis fungoides with an oral aromatic retinoid (Ro 10-9359). In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 313. Springer-Verlag, New York.

Spielvogel, R. L., DeVillez, R. L., and Roberts, L. C. (1985): Oral isotretinoin therapy for familial Muir-Torre syndrome. *J. Am. Acad. Dermatol.* 12:475–480.

Stadler, R., Marcelo, C. L., Voorhees, J. J., and Orfanos, C. E. (1984): Effect of a new retinoid, arotinoid (Ro 13-6298), on *in vitro* keratinocyte proliferation and differentiation. *Acta Derm. Venereol. (Stockh)* 64:405–411.

Stern, R. S., Rosa, F., and Baum, C. (1984): Isotretinoin and pregnancy. *J. Am. Acad. Dermatol.* 10:851–854.

Stewart, M. E., Benoit, A. M., Stranieri, A. M., Rapini, R. P., Strauss, J. S., and Downing, D. T. (1983): Effect of oral 13-*cis*-retinoic acid at three dose levels on sustainable rates of sebum secretion and on acne. *J. Am. Acad. Dermatol.* 8:532–538.

Stewart, M. E., Benoit, A. M., Downing, D. T., and Strauss, J. S. (1984): Suppression of sebum secretion with 13-*cis*-retinoic acid: effect on individual skin surface lipids and implications for their anatomic origin. *J. Invest. Dermatol.* 82:74–78.

Stollenwerk, R., Fischer-Hoinkes, H., Komenda, K., and Schilling, F. (1981): Clinical observations on oral retinoid therapy of psoriatic arthropathy (Ro 10-9359). In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 205. Springer-Verlag, New York.

Strauss, J. S., Stranieri, A. M., Farrell, L. N., and Downing, D. T. (1980): The effect of marked inhibition of sebum production with 13-cis-retinoic acid on skin surface lipid composition. *J. Invest. Dermatol.* 74:66–67.

Strauss, J. S., Rapini, R. P., Shalita, A. R., Konecky, E., Pochi, P. E., Comite, H., and Exner, J. H. (1984): Isotretinoin therapy for acne: results of a multicenter dose-response study. *J. Am. Acad. Dermatol.* 10:490–496.

Street, M. L., White, J. W., Jr., and Gibson, L. E. (1990): Multiple keratoacanthomas treated with oral retinoids. *J. Am. Acad. Dermatol.* 23:862–866.

Stumpenhausen, G., Hein, R., Kulozik, M., Mauch, C., Bryce, G. F., Oono, T., and Krieg, T. (1991): The influence of retinoids on fibroblast functions. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 139–150. Karger, Basel, New York.

Stuttgen, G. (1975): Oral vitamin A acid therapy. *Acta Derm. Venereol. (Stockh)* 55(Suppl 74):174–179.

Sulik, K. K., and Alles, A. J. (1991): Teratogenicity of the retinoids. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 282–295. Karger, Basel, New York.

Tamayo, L., and Ruiz-Maldonado, R. (1981): Long-term follow-up of 30 children under oral retinoid Ro 10-9359. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. pp. 287–294. Springer-Verlag, New York.

Tangrea, J., Edwards, B., Hartman, A., Taylor, P., Peck, G., Salasche, S., Menon, P., Winton, G., Mellette, R., Guill, M., Robinson, J., Guin, J., and Stoll, H. (1990): Isotretinoin-basal cell carcinoma prevention trial. Design, recruitment results, and baseline characteristics of the trial participants. The ISO-BCC Study Group. *Controlled Clin. Trials* 11:433–450.

Tangrea, J. A., Edwards, B. K., Taylor, P. R., Hartman, A. M., Peck, G. L., Salasche, S. J., Menon, P. A., Benson, P. M., Mellette, J. R., Guill, M. A., Robinson, J. K., Guin, J. D., Stoll, H. L., Grabski, W. J., Winston, G. B., and Isotretinoin-Basal Cell Carcinoma Study Group (1992a): Long-term therapy with low-dose isotretinoin for prevention of basal cell carcinoma: a multicenter clinical trial. *J. Natl. Cancer Inst.* 84:328–332.

Tangrea, J. A., Kilcoyne, R. F., Taylor, P. R., Helsel, W. E., Adrianza, M. E., Hartman, A. M., Edwards, B. K., and Peck, G. L. (1992b): Skeletal hyperostosis in patients receiving chronic, very-low-dose isotretinoin. *Arch. Dermatol.* 128:921–925.

Teelmann, K. (1981): Experimental toxicology of the aromatic retinoid Ro 10-9359 (etretinate). In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 41. Springer-Verlag, New York.

Thomson, J., and Milne, J. A. (1969): The use of retinoic acid in congenital ichthyosiform erythroderma. *Br. J. Dermatol.* 81:452–455.

Tong, P. S., Horowitz, N. N., and Wheeler, L. A. (1990): *Trans*-retinoic acid enhances the growth response of epidermal keratinocytes to epidermal growth factor and transforming growth factor beta. *J. Invest. Dermatol.* 94:126–131.

Törma, H., and Vahlquist, A. (1984): Vitamin A uptake by human skin *in vitro*. *Arch. Dermatol. Res.* 276:390–395.

Török, L., Galuska, L., Kasa, M., and Kadar, L. (1989): Bone-scintigraphic examinations in patients treated with retinoids: a prospective study. *Br. J. Dermatol.* 120:31–36.

Vahlquist, A., and Torma, H. (1991): Metabolic interactions between synthetic and natural retinoids. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 69–78. Karger, Basel, New York.

Vahlquist, A., and Törma, H. (1988): Retinoids and keratinization. Current concepts. *Int. J. Dermatol.* 27:81–95.

Vahlquist, C. (1991): Acitretin and blood lipids. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. pp. 296–301. Karger, Basel, New York.

Van der Rhee, H. J., and Polano, M. K. (1981): Treatment of psoriasis vulgaris with a low-dosage Ro 10-9359 orally combined with corticosteroids topically. In: *Retinoids: Advances in basic research and therapy*, edited by C. E. Orfanos, O. Braun-Falco, E. M. Farber, C. Grupper, M. K. Polano, and R. Schuppli. p. 193. Springer-Verlag, New York.

van Dooren-Greebe, R. J., van de Kerkhof, P. C., Chang, A., and Happle, R. (1989): Acitretin monotherapy in acrodermatitis continua Hallopeau. *Acta Derm. Venereol. (Stockh)* 69:344–346.

Varani, J., Mitra, R. S., Gibbs, D., Phan, S. H., Dixit, V. M., Mitra, R., Jr., Wang, T., Siebert, K. J., Nickoloff, B. J., and Voorhees, J. J. (1990): All-trans retinoic acid stimulates growth and extracellular matrix production in growth-inhibited cultured human skin fibroblasts. *J. Invest. Dermatol.* 94:717–723.

Verma, A. K., Shapas, B. G., Rice, H. M., and Boutwell, R. K. (1979): Correlation of the inhibition by retinoids of tumor promoter-induced mouse epidermal ornithine decarboxylase activity and of skin tumor promotion. *Cancer Res.* 39:419–425.

Verma, A. K., and Boutwell, R. K. (1977): Vitamin A acid (retinoic acid), a potent inhibitor of 12-*O*-tetradecanoyl-phorbol-13-acetate-induced ornithine decarboxylase activity in mouse epidermis. *Cancer Res.* 37:2196–2201.

Viallet, J. P., Ruberte, E., du Manoir, S., Krust, A., Zelent, A., and Dhouailly, D. (1991): Retinoic acid-induced glandular metaplasia in mouse skin is linked to the dermal expression of retinoic acid receptor beta mRNA. *Dev. Biol.* 144:424–428.

Wang, C. C., Straight, S., and Hill, D. L. (1976): Destabilization of mouse liver lysosomes by vitamin A compounds and analogues. *Biochem. Pharmacol.* 25:471–475.

Ward, A., Brogden, R. N., Heel, R. C., Speight, T. M., and Avery, G. S. (1983): Etretinate. A review of its pharmacological properties and therapeutic efficacy in psoriasis and other skin disorders. *Drugs* 26:9–43.

Ward, P. S., and Jones, R. D. (1989): Successful treatment of a harlequin fetus. *Arch. Dis. Child* 64:1309–1311.

Weinstein, R. S., Merk, F. B., and Alroy, J. (1976): The structure and function of intercellular junctions in cancer. *Adv. Cancer Res.* 23:23–89.

Weiss, V. C., West, D. P., Ackerman, R., and Robinsón, L. A. (1984): Hepatotoxic reactions in a patient treated with etretinate. *Arch. Dermatol.* 120:104–106.

West, M. R., Page, J. M., Turner, D. M., Wood, E. J., Holland, D. B., Cunliffe, W. J., and Rupniak, H. T. (1992): Simple assays of retinoid activity as potential screens for compounds that may be useful in treatment of psoriasis. *J. Invest. Dermatol.* 99:95–100.

White, S. I., and MacKie, R. M. (1989): Bone changes associated with oral retinoid therapy. *Pharmacol. Ther.* 40:137–144.

Wiegand, U.-W., and Jensen, B. K. (1991): Pharmacokinetics of acitretin in humans. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. p. 192. Karger, Basel, New York.

Williams, M. L., and Elias, P. M. (1981): Nature of skin fragility in patients receiving retinoids for systemic effect. *Arch. Dermatol.* 117:611–619.

Wilson, E. L., and Reich, E. (1978): Plasminogen activator in chick fibroblasts: induction of synthesis by retinoic acid; synergism with viral transformation and phorbol ester. *Cell* 15:385–392.

Wozel, G., Chang, A., Zultak, M., Czarnetzki, B. M., Happle, R., Barth, J., and van de Kerkhof, P. C. (1991): The effect of topical retinoids on the leukotriene-B4-induced migration of polymorphonuclear leukocytes into human skin. *Arch. Dermatol. Res.* 283:158–161.

Wright, A. L., Gawkrodger, D. J., Branford, W. A., McLaren, K., and Hunter, J. A. A. (1988): Self-healing epitheliomata of Ferguson-Smith: cytogenetic and histological studies, and the therapeutic effect of etretinate. *Dermatologica* 176:22–28.

Zachariae, H., and Thestrup-Pedersen, K. (1990): Interferon alpha and etretinate combination treatment of cutaneous T-cell lymphoma. *J. Invest. Dermatol.* 95:206S–208S.

Zech, L. A., Gross, E. G., Peck, G. L., and Brewer, H. B. (1983): Changes in plasma cholesterol and triglyceride levels after treatment with oral isotretinoin. A prospective study. *Arch. Dermatol.* 119:987–993.

Zheng, Z.-S., Polakowska, R., Johnson, A., and Goldsmith, L. A. (1992): Transcriptional control of epidermal growth factor receptor by retinoic acid. *Cell Growth Differ.* 3:225–232.

Zile, M. H., Schnoes, H. K., and DeLuca, H. F. (1980): Characterization of retinoyl beta-glucuronide as a minor metabolite of retinoic acid in bile. *Proc. Natl. Acad. Sci. U.S.A.* 77:3230–3233.

Zouboulis, C. C., Xia, L., Korge, B., Gollnick, H., and Orfanos, C. E. (1991): Cultivation of human sebocytes *in vitro*: cell characterization and influence of synthetic retinoids. In: *Retinoids: 10 years on*, edited by J.-H. Saurat. p. 254–273. Karger, Basel, New York.